Research Report

Effects of Peritoneal Fluid on Sperm Motility and Viability in Endometriosis

Pengaruh Cairan Peritoneal pada Motilitas Sperma dan Viabilitas pada Endometriosis

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Abstract

Objective: To know the effects of peritoneal fluid on sperm motility and viability in patients with endometriosis.

Design/data identification: This was a laboratory experimental study to peritoneal fluid from endometriosis and non-endometriosis patients who underwent surgery in Dr. Hasan Sadikin Hospital and around with endoscopy facility which fulfill inclusion and exclusion criterias. Experiments was performed in ASTER Fertility Clinic, Dr. Hasan Sadikin Hospital.

Method: Semen samples were normozoospermic of which has been prepared using swim up method with sperm count 3 x 10^6/ml. The sperm were exposed to peritoneal fluid from endometriosis and non-endometriosis patients and analyzed at h 0, 1, 3, 6, and 24 to see the difference of sperm motility and viability postincubation with endometriosis peritoneal fluid. The sperm viability was detected using trypan blue 0.4%.

Result: Exposure of sperm to peritoneal fluid reduced sperm motility significantly from the h 6 observation (Zw = 2.17; p = 0.03) and the h 24 (Zw = 2.35; p = 0.01). The sperm viability which incubated with endometriosis peritoneal fluid reduced significantly from h 6 observation (Zw = 1.99; p = 0.04) and the h 24 (Zw = 2.55; p = 0.01).

Conclusion: The endometriosis peritoneal fluid reduced the motility and viability of the sperm began from the h 6 postincubation. This indicate the possibility of involvement of endometriosis peritoneal fluid to infertility.

[Indones J Obstet Gynecol 2011; 35-1: 18-20]

Keywords: sperm motility, sperm viability, endometriosis

Abstrak

***Tujuan**: Untuk mengetahui pengaruh cairan peritoneum pasien dengan endometriosis terhadap penurunan motilitas dan viabilitas sperma.*

***Rancangan/rumusan data**: Penelitian ini merupakan penelitian eksperimental laboratoris yang dilakukan terhadap cairan peritoneum pasien endometriosis dan pasien bukan endometriosis yang menjalani operasi laparoskopi atau laparotomi di Rumah Sakit Dr. Hasan Sadikin dan rumah sakit yang memiliki fasilitas laparoskopi dan memenuhi kriteria inklusi dan eksklusi. Eksperimen dilakukan di Laboratorium Infertilitas ASTER RS Dr. Hasan Sadikin.*

***Metode**: Sperma yang digunakan adalah sperma yang dipreparasi di Laboratorium Infertilitas ASTER RS Dr. Hasan Sadikin dengan metode swim up sehingga diperoleh sperma berjumlah 3 x 10^6/ml. Sperma tersebut kemudian diinkubasikan dengan cairan peritoneum pasien endometriosis dan tanpa endometriosis. Pemantauan motilitas dan viabilitas sperma dilakukan dengan metode manual pada jam ke 0, 1, 3, 6, 24 pascainkubasi untuk mengetahui perubahan motilitas dan viabilitas sperma. Pemeriksaan viabilitas dilakukan dengan menggunakan pewarnaan trypan blue 0,4%.*

***Hasil**: Motilitas sperma pasien endometriosis menurun secara bermakna pada pemeriksaan mulai jam ke-6 pascainkubasi (Zw = 2,17; p = 0,03) hingga jam ke-24 pascainkubasi (Zw = 2,35; p = 0,019). Viabilitas sperma pasien endometriosis menurun secara bermakna pada pemeriksaan mulai jam ke-6 (Zw = 1,994; p = 0,04) hingga jam ke-24 pascainkubasi. (Zw = 2,55; p = 0,01).*

***Kesimpulan**: Cairan peritoneum pasien endometriosis menurunkan motilitas dan viabilitas sperma mulai jam ke-6 pascainkubasi. Hal ini dapat mengindikasikan kemungkinan pengaruh cairan peritoneum endometriosis terhadap infertilitas.*

[Maj Obstet Ginekol Indones 2011; 35-1: 18-20]

***Kata kunci**: motilitas sperma, viabilitas sperma, endometriosis*

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INTRODUCTION

Endometriosis is a disease that frequently associated with infertility or a decrease in fecundity though its mechanisms is still a controversion.[1-4] Fecundity is defined as the probability of a woman achieving a live birth for any given month. In normal couples, fecundity is in the range of 0.15 to 0.20 per month and decreases with age. In untreated women with endometriosis and infertility, monthly fecundity is 0.02 to 0.10.[2] One of the presume of endometriosis-related infertility was the decrease of sperm motility due the content of the endometriosis peritoneal fluid which is different from non-endometriosis peritoneal fluid.[5-8]

Peritoneal fluid has been a focus of research on endometriosis because of the extent of information it potentially carries about the disease. The milieu in which the immune mediators associated with the local inflammation of endometriosis can be studied in order to see the relation between endometriosis and infertility. Several authors have suggested that peritoneal from endometriosis inhibits sperm motility.[9] This contrasts with the findings of other studies.[7,10,11] All of those studies was conducted using computer-aided sperm analysis (CASA).

The prevalence of pelvic endometriosis widely vary. It is also a reproductive health problem in Indo-

nesia, since it plays role as one of the cause of infertility. A hospital-based research in Dr Sutomo Hospital, Surabaya stated that incidency of endometriosis in infertility group is 37.2%.[12] The disorder is most commonly diagnosed in women of reproductive age, although it was a difficult disease to diagnose because of variability in symptoms and signs and confusion with other disorders. The current clinical opinion is that a surgical procedure such as laparoscopy is required for definitive diagnosis of endometriosis.[2] Laparoscopy is the gold standard diagnostic test in clinical practice.[13]

The purpose of the study was to prove that endometriosis peritoneal fluid effected on decreasing sperm motility and viability.

MATERIALS AND METHODS

Collection and Preparation of Peritoneal Fluid

Peritoneal fluid was collected from the pouch of Douglas of patients scheduled for laparoscopy for various indications. Two groups of patients were identified: (1) Patients with no visible endometriosis (n = 13); (2) Patients with endometriosis (n = 13). Peritoneal fluids were aspirated into sterile plastic tubes, and transported to the laboratory. Peritoneal fluids were centrifuged for 10 minutes at 1500 rpm to remove cellular debris. Samples were filtered through a 0.22µ membrane (Sartorius Stedim®, Biotech, Denmark) and stored at -70°C until use. At the time of the experiment, samples of peritoneal fluid were brought to room temperature.

Preparation of Sperm Samples

Semen samples were obtained from 13 normozoospermic donors. The samples were collected by masturbation after 3 to 7 days of sexual abstinence. After complete liquefaction, semen analysis was performed according to World Health Organization guidelines and morphology strict criteria.[14] The total number of samples used is the same as the number of experiments performed in each study. The sperm was prepared using swim up techniques and the sperm concentration was adjusted to 3 x 10^6/ml.

Peritoneal Fluid-Sperm Interaction

One hundred microliters of the sperm suspension was mixed in a sterile Eppendorf tube with the following 2 groups: group 1, 100 µl peritoneal fluid from patients without endometriosis; group 2, 100 µl peritoneal fluid from patients with endometriosis. The mixtures were incubated in 37°C, 6% CO_2 environment and analyzed at 0, 1, 3, 6, and 24 h of incubation. On each time point the following parameters were measured percentage motility and viability.

Statistical Analysis

Statistical analysis employed Wilcoxon signed-rank test using the SPSS computer package (Release 13.0, SPSS, Chicago, IL, USA) to compare sperm motility and viability in women without and with endometriosis. The normality test was analyzed using Saphiro-Wilk test. Parameters were expressed as a percentage. The statistical significance of differences was considered significant if p value was < 0.05.

RESULTS

Exposure of sperm to peritoneal fluid reduced sperm motility significantly from the h 6 observation (Zw = 2.17; p = 0.03) and the h 24 (Zw = 2.35; p = 0.01).

Table 1. Sperm Motility Postincubation with Non-Endometriosis and Endometriosis Peritoneal Fluid.

Motility (%)	Sperm + Non-Endometriosis Peritoneal Fluid n = 13	Sperm + Endometriosis Peritoneal Fluid n = 13	Zw	p value
h-0				
Median	92	92	0.00	1.00
Range	73 - 99	73 - 99		
h-1				
Median	91	91	0.95	0.34
Range	72 - 97	60 - 97		
h-3				
Median	85	85	1.63	0.10
Range	23 - 92	5 - 92		
h-6				
Median	85	73	2.17	0.03
Range	45 - 92	2 - 91		
h-24				
Median	39.0	1	2.35	0.01
Range	1 - 79	0 - 59		

Note: Zw = Wilcoxon signed-rank test

The sperm viability which incubated with endometriosis peritoneal fluid reduced significantly from h 6 observation (Zw = 1.994; p = 0.04) and the h 24 (Zw = 2.55; p = 0.01).

Table 2. Sperm Viability Postincubation with Non-Endometriosis and Endometriosis Peritoneal Fluid.

Viability	Sperm + Non-Endometriosis Peritoneal Fluid n = 13	Sperm + Endometriosis Peritoneal Fluid n = 13	Zw	p value
h-0				
Median	100	99	1.63	0.10
Range	97 - 100	90 - 100		
h-1				
Median	99	98	1.572	0.11
Range	90 - 100	70 - 100		
h-3				
Median	93	92	0.86	0.38
Range	82 - 98	61 - 99		
h-6				
Median	90	86	1.994	0.04
Range	82 - 95	60 - 94		
h-24				
Median	80	72	2.55	0.01
Range	65 - 95	50 - 92		

DISCUSSION

This study showed that there were significant decreased in sperm motility and viability which incubated in endometriosis peritoneal fluid started h-6 postincubation. This result was different from other studies.[7,9] This study was applying percentage to see the decline of sperm motility (progressive motility) and viability, whereas other study applied the decline of sperm swimming velocity which micrometer per second. The postincubation viability assessed by mixing one drop of sperm suspension with Trypan blue 0.4% and examined at x 400 magnification by using a microscope. Trypan blue 0.4% will put blue color in a nonviable sperm. Other study applying Eosin Y to the sperm suspension.[7] Both solutions was written on WHO laboratory manual,[14,15] so the researcher may use the kind that available in the institution.

Some studies have shown the influence of peritoneal fluid towards sperm function with different results.[7,9-11,16-18]Munuce et al[7] suggested that exposure of sperm to peritoneal fluid for up to 6 hours did not affect sperm viability or motility. On that study the motility examination was done on h 0, 2, and 6 hours postincubation applying CASA which can examined the curvilinear velocity, progressive velocity, percentage of linearity, and lateral head displacement. Aeby et al.[9] suggested a similar result with Munuce et al.[7] Aeby suggested that the decrease of sperm motility and viability was found in stage I endometriosis (minimal endometriosis) which also evaluated using CASA. This study applying manual sperm analysis. This method have both advantage and disadvantage. The advantage of using this method is to avoid difficulties in distinguishing spermatozoa from particulate debris which often occur in CASA method,[14,15] whereas the disadvantage of the manual analysis is only able to differenciate progressive, nonprogressive, and immotile.

Sperm life span to execute fertilization in woman body is approximately 48 - 72 hours. Most pregnancy occures if the intercourse take place during 3 days interval before ovulation.[19] However, action should be made to raise the fecundity rate in endometriosis patient besides definitive treatment knowing that the sperm motility and viability decreased significantly after 6 hours in patient with endometriosis.

CONCLUSIONS AND SUGGESTION

In conclusion, we have demonstrated that the endometriosis peritoneal fluid reduced the motility and viability of the sperm began from the h 6 postincubation. This indicate the possibility of involvement of endometriosis peritoneal fluid to infertility. We suggest to perform follicles observation closely in endometriosis patients so the duration of ovulation and intercourse less than 6 hours in order to increase the fecundity rate.

Acknowledgement: The authors thank dr. Wachyu Hadisaputra, SpOG(K), Department of Obstetrics and Gynecology, Indonesia University School of Medicine, Jakarta and dr. Nadir Chan, SpOG(K), YPK Hospital, Jakarta.

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