Effects of Peritoneal Fluid on Sperm Motility and Viability in Endometriosis

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Abstract

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

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 from the h 6 postincubation. This indicate the possibility of involvement of endometriosis peritoneal fluid to infertility.

Keywords: sperm motility, sperm viability, endometriosis

INTRODUCTION

Endometriosis is a disease that frequently associated with infertility or a decrease in fecundity though its mechanisms is still a controversy.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 presumes 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 fluid 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 incidence of endometriosis in infertility group is 37.2%.\textsuperscript{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.\textsuperscript{2} Laparoscopy is the gold standard diagnostic test in clinical practice.\textsuperscript{13}

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

\section*{MATERIALS AND METHODS}

\subsection*{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 0.22 $\mu$m membrane (Sartorius Stedim\textsuperscript{®}, Biotech, Denmark) and stored at -70°C until use. At the time of the experiment, samples of peritoneal fluid were brought to room temperature.

\subsection*{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.\textsuperscript{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 $3 \times 10^6$/ml.

\subsection*{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 $\mu$l peritoneal fluid from patients without endometriosis; group 2, 100 $\mu$l 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.

\subsection*{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 Sapiro-Wilk test. Parameters were expressed as a percentage. The statistical significance of differences was considered significant if $p$ value was $< 0.05$.

\section*{RESULTS}
Exposure of sperm to peritoneal fluid reduced sperm motility significantly from the h 6 observation ($Z_{w} = 2.17; p = 0.03$) and the h 24 ($Z_{w} = 2.35; p = 0.01$).

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Motility (%)} & \textbf{Sperm + Non-Endometriosis Peritoneal Fluid n = 13} & \textbf{Sperm + Endometriosis Peritoneal Fluid n = 13} & \textbf{Zw} \textbf{ p value} \\
\hline
\hline
h-0 Median & 92 & 92 & 0.00 \ 1.00 \\
Range & 73 - 99 & 73 - 99 & \\
\hline
h-1 Median & 91 & 91 & 0.95 \ 0.34 \\
Range & 72 - 97 & 60 - 97 & \\
\hline
h-3 Median & 85 & 85 & 1.63 \ 0.10 \\
Range & 23 - 92 & 5 - 92 & \\
\hline
h-6 Median & 85 & 73 & 2.17 \ 0.03 \\
Range & 45 - 92 & 2 - 91 & \\
\hline
h-24 Median & 39.0 & 1 & 2.35 \ 0.01 \\
Range & 1 - 79 & 0 - 59 & \\
\hline
\end{tabular}
\caption{Sperm Motility Postincubation with Non-Endometriosis and Endometriosis Peritoneal Fluid.}
\end{table}

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

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Viability} & \textbf{Sperm + Non-Endometriosis Peritoneal Fluid n = 13} & \textbf{Sperm + Endometriosis Peritoneal Fluid n = 13} & \textbf{Zw} \textbf{ p value} \\
\hline
\hline
h-0 Median & 100 & 99 & 1.63 \ 0.10 \\
Range & 97 - 100 & 90 - 100 & \\
\hline
h-1 Median & 99 & 98 & 1.572 \ 0.11 \\
Range & 90 - 100 & 70 - 100 & \\
\hline
h-3 Median & 93 & 92 & 0.86 \ 0.38 \\
Range & 82 - 98 & 61 - 99 & \\
\hline
h-6 Median & 90 & 86 & 1.994 \ 0.04 \\
Range & 82 - 95 & 60 - 94 & \\
\hline
h-24 Median & 80 & 72 & 2.55 \ 0.01 \\
Range & 65 - 95 & 50 - 92 & \\
\hline
\end{tabular}
\caption{Sperm Viability Postincubation with Non-Endometriosis and Endometriosis Peritoneal Fluid.}
\end{table}
DISCUSSION
This study showed that there were significant decreases 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 studies 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 on a nonviable sperm. Other study applying Eosin Y to the sperm suspension.7 Both solutions was written in a nonviable sperm. Other study applying Eosin Y 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 differentiate progressive, nonprogressive, and immotile.

Sperm life span to execute fertilization in woman body is approximately 48 - 72 hours. Most pregnancy occurs if the intercourse take place during 3 days interval before ovulation.19 However, action should be made to raise the fecundity rate in endometriosis. Fertil Steril. 1996: 174

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.

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