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Toxic and Non-Toxic Peritoneal Fluid:Effects on Sperm Motility

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불임환자의 복강내액이 정자 활동성에 미치는 영향

제일병원 산부인과

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〔요 약〕

불임여성에서 복강내액의 독성 유무를 알기 위해서 콤퓨터 정액 분석기를 이용하여 복강내액의 정자 활동성을 분석하였다. 연구대상은 자궁내막증 환자 14예와 자궁내막증이 없는 불임환자로부터의 복강내액 9예로 하였다. 연구방법은 건강인의 정자를 세척 분리하여 대상환자의 복강내액과 Hams F10 배양액을 동량 혼합하여(50%) 배양후 정자 활동성을 측정하였다. 정자 활동성의 감소는 배양전 0시간의 수치와 비교해서 1시간, 4시간 그리고 24시간후의 결과를 비교 분석하였다. 자궁내막증 제1기 및 제2기 환자와 자궁내막증이 없는 환자의 복수에서의 정자 활동성의 감소는 통계학적으로 의의있는 차이는 없었다. 그러나 자궁내막증이 심한 제3기의 환자군에서는 의의있는 차이가 있었다($p<.05$). 실험군간의 정자 활동성의 평균치 차이는 없었으나, 각 군에서의 환자별 개인 성적은 정자 활동성이 현저히 감소한 독성있는 복강내액과 비독성의 복강내액을 쉽게 구별할 수 있었다. 결론적으로 정자 배양을 이용한 복강내액 독성 검사는 비교적 간단하고 경제적이므로 임상적으로 최근에 대두된 복강내 수정 치료법의 예비검사로서 유용할 것으로 사료된다.

INTRODUCTION

The peritoneal fluid(PF) microenvironment is an important zone of gamete interaction, sensitive to the influences of pathologic conditions and possessing constituents capable of impacting on reproductive processes. Endometriosis-associated infertility may be mediated by the detrimental effect of PF factors on spermatozoa (Oak et al. 1985), oocytes(Chacho et al. 1986), or embryos(Sims et al. 1988;Prough et al. 1988). Whether minimal endometriosis causes infertility is still controversial(Wheeler & Malinak, 1988). Therefore it seems meaningful to determine whether PF from women with endometriosis might have toxic effects on sperm function. Recently, direct intraperitoneal insemination(DIPI) has been introduced as a alternative treatment for unexplained infertility, cervical dysfunction, immunologic infertility, and maleinfertility(Forrler et al. 1986). The conditions necessary for a successful pregnancy after DIPI include sperm survival in PF, occurrence of ovulation, sperm and ovum pick-up by the tube, and zygote transport to the uterus. To fulfill the these criteria, first of all, sperm must be able to survive in the peritoneal cavity.

This study used computer-assisted semen analysis(CASA) to evaluate the effects of PFs on sperm motility, We evaluated PFs from patients with and without endometriosis.

MATERIALS AND METHODS

Semen Preparation

Semen samples from 3 healthy donors were collected by masturbation after at last 2 days of

abstinence and were examined within 30 to 60 minutes after ejaculation. The specimens were processed using an overlay technique to collect the motile sperm atrer swim-up. The samples were washed twice with BWW medium by centrifugation at 250×g for 7 minutes, diluted to 20 million sperm/ml, and incubated for 60 minutes at 37℃ in 5% CO_2 to allow viable sperm to swim up into the supernatant. Motile sperm were pended in BWW with or without 3.5% bovine serum albumin(BSA) and aliquoted into 96 well trays filled with 1:1(vol/vol) 100μl of PF. The concentration of PF was diluted to 50 % with BWW in each well. All samples were maintained at 37℃ in CO_2 incubator during the test period.

Peritoneal Fluid Preparation

PF from the cul-de-sac was obtained from 23 women with or without endometriosis. For 20/23 women the PFs were collected during diagnostic laparoscopy. For 2/23 women the PFs were obtained during laparoscopy for tubal ligation. In one case the PF was obtained during laparotomy for microsurgery. All samples were centrifuged at 500×g for 10 minutes, and the supernatant was frozen until assay. The PF was filtered through a 0.22μm sterile filter (Millipore Corp., Bedford, NJ) before being added to the assay. Half of the each sample was heat inactivated at 56℃ for 30 minutes.

Computer-Assisted Semen Analysis(CASA)

A potion of sperm specimen(5μl) was placed into Makler chamber(Sefi-Medical Instruments, Israel) to determine the percentage of motility, linearity, velocity, lateral head displacement (LHD), and beat frequency. These parameters were measured by CASA(Cell soft, CryoResourecs Ltd., New York). The concentration of washed sperm was adjusted to 20×10^6/ml from 40×10^6/ml before analysis to reduce the coefficient of variation and to limit sperm collision. More than 100 sperm were counted for each assessment(Vantman et al. 1988).

Experimental Design

Well suspended swirn-up sperm were incubated with PF. Sperm cultured in 100% BWW medium without PF served as a control. PFs from endometriosis patients(n=14) and patients without endometriosis(n=9) were evaluated.

Study subjects were divided into 5 groups: group 1 in BWW medium only(n=5), group 2 in PF from patients without endometriosis(n=9), group 3 in PF from endometriosis stage Ⅰ patients(n=6), group 4 in PF from stage Ⅱ patients(n=4), and group 5 in PF from stage Ⅲ patients(n=4). Toxicity was defined as a decreased of >30% in 1 hour or 4 hours or >50 % after 24 hour incubation. Classification of endometriosis proposed by the American Fertility Society(AFS) was used in all cases(Fertil & Steril, 1985).

Statistical Analysis

Statistical analysis was done by the one way-analysis of variance(ANOVA) with Duncan's multiple range test for variables. Data were considered statistically significant when the value was <0.05.

RESULTS

To assess the detrimental effect of PF on sperm motility, the percentage of motile sperm of each sample was evaluated after 1, 4 and 24 hours of incubation, and subtracted from the percent of motility at tine 0(immediately after mixing with PF). The decrement in the percentage of motile sperm is shown in Figure 1. No Statistically difference was noted in the mean motility decrement between groups 1, 2, 3, 4 and 5 except at 24 hour in group 5($p<.05$).

Although with wide standard deviations, each subject in all groups showed bimodal distrbution (Fig. 2) and we could clearly differentiate toxic and non-toxic PFs($p<.001$), which were distributed in each group except in group 4, including PFs from the patients without endometriosis. According to the criteria of toxic PF, we defined each individual as toxic or nontoxic, the analyzed statistically. There were highly significant differences in the decrement of motility per-

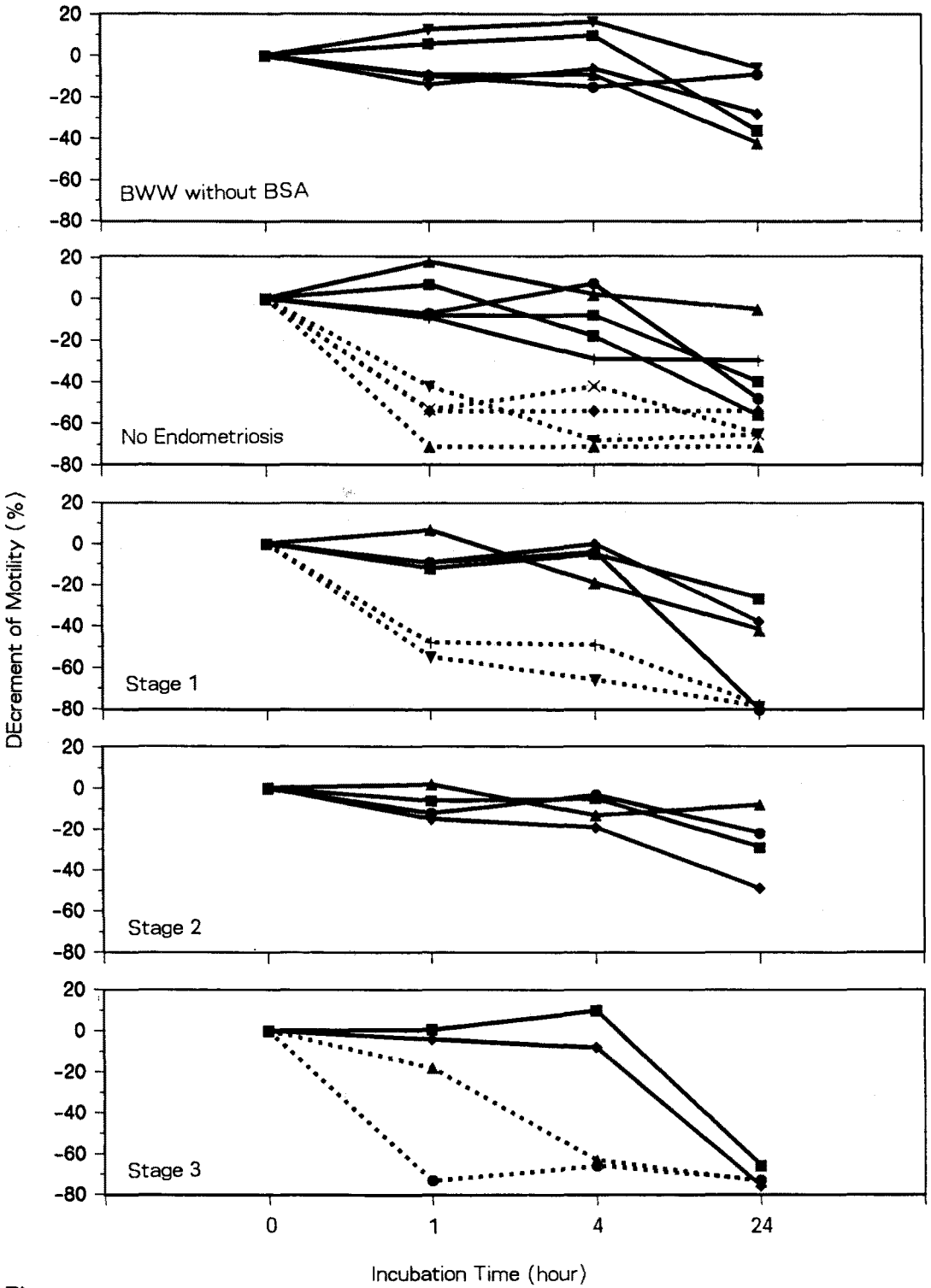

Fig. 1. The decrement in the percentage of motile sperm after 1, 4 and 24 hours of incubation, subtracted from the percent of motility at time 0.

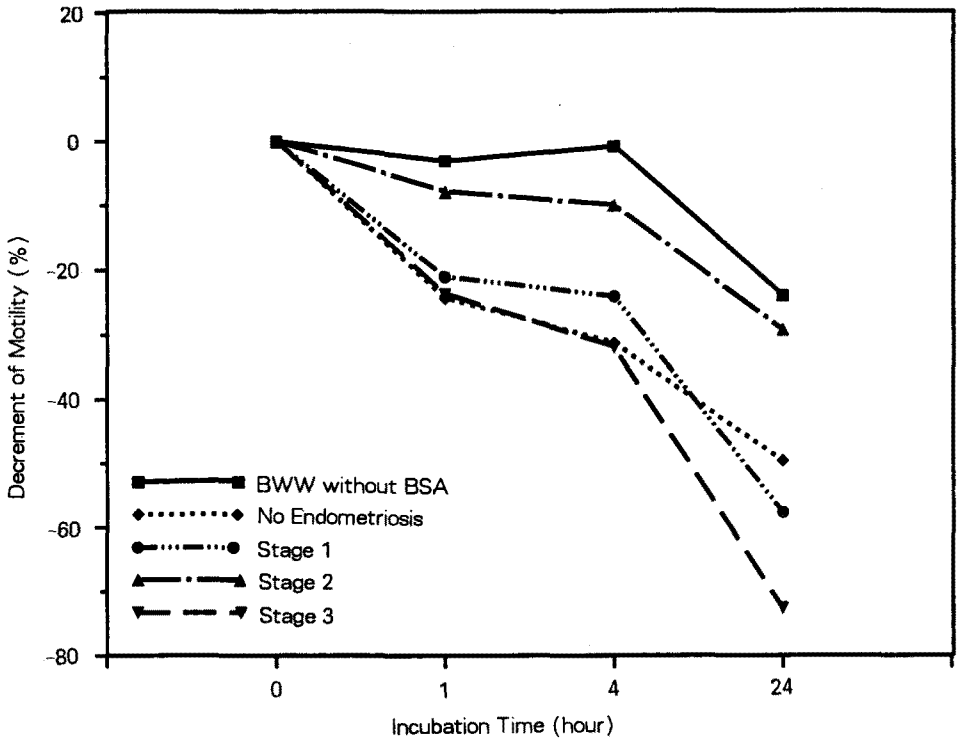

Fig. 2. Individualized data of motility decrement in all groups. Toxic PF(---) and nontoxic PF(—) could be differentiated($p<.001$).

centage between toxic and nontoxic PFs in the groups($p<.001$).

Simple linear regression analysis of the transformed data was employed to access the relation between motility and velocity, linearity, LHD and beat cross frequency. The relationships were evaluated for 1, 4 and 24 hours post-treatment of the sperm. There was a strong correlation between motility and velocity($r=0.8$), LHD($r=0.6$) and beat cross frequency($r=0.7$). Motility and linearity were not found to correlate($r=0.2$).

To evaluate the role of heat inactivation, we duplicated the procedures with heat-inactivated PF. The results obtained were nearly indentical to those found without heat-inactivation; there was no statistical difference between groups 2, 3, 4 and 5, except at 24 hours in group 5($p<.05$). We also evaluated the effect of adding BSA to the culture medium. Although BSA seemed to increase sperm motility slightly, there was no significant difference(data not shown).

DISCUSSION

Local peritoneal factors and their role in infertility associated with endometriosis is an area of high interest(Awadalla et al. 1987). Infertility in the presence of mild endometriosis in difficult to explain. Suggestions have been made that pelvic endometriosis may cause infertility by interfering with sperm motility or transport, or by increasing the phagocytosis of sperm by intraperitoneal macrophages(Muscato et al. 1981). In addition, PF from endometriosis patients may cause devastating effects on sperm penetration and mouse embryo development (Sims et al. 1988; Prough et al. 1988). In contrast, mild endometriosis dose not affect sperm transport or survival, dose not increase sperm phagocytosis or affect embryo development (Awadalla et al. 1987; Stone & Himsl, 1986). The rate of sperm recovery at laparoscopy following artificial insemination is similar in pati-

ents with and without minimal endometriosis (AFS Stage 1). Therefore the cause of infertility in patients in the early stage of endometriosis remains obscured.

We found that toxic PF could be clearly differentiated. Individuals with toxic PF were in each group, even the control group(group 2: those without endometrisosis). Only group 4 (mild endometriosis) was exempt from this observation:all had non-toxic PF. A significant reduction in the percentage of motile sperm in women with unexplained infertility and infertile women with endometriosis has been reported (Oak et al. 1985). Our study revealed individualized reduction of sperm motility that was not related to the presence or severity of endometriosis.

The finding that linearity was not also correlated with sperm motility is surprising, since the other variables associated with sperm viability were also correlated with motility. The data did not indicate that the sperm were undergoing hyperactivation or other swimming pattern changes often associated with sperm incubation in culture.

It is not clear what kind of soluble factors(acellular components of PF) are involved with toxicity. Recently, a significant reduction of sperm motility in vitro was reported to be induced by the tumor necrosis factor(TNF) found in PF of women with minimal endometriosis (Eisermann et al. 1988).

The presence of TNF in PF is associated with **primary infertility and endometrisosi. The proportion of PF INF-positive women with pelvic inflammatory disease(PID) and those with moderate** and severe endometriosis was significantly higher than in women with normal pelvic anatomy(Eisermann et al. 1988).

Lymphocyte proliferation in the absence of mitogen is significantly higher in PFs from women with unexplained infertility. Such samples had significantly elevated levels of interleukin-2;none of the PF samples from fertile controls demonstrated elevated levels of these cytokines(Hill & Anderson, 1988). Therefore, measurement of these factor(TNF, Interleukin-1, and Interleukin-2) may be valuable in evaluating toxic PFs determined by sperm incubation tests.

Although some published findings could not be duplicated by others(Redwine, 1988), invisible endometriosis was found electromicroscopically in 25% of random biopsy specimens of visually normal peritoneums in 20 patients with endometriosis(Murphy et al. 1986). We could differentiate toxic and non-toxic PFs, not only from patients with endometriosis but also from infertile women without endometriosis. Therefore it could be postulated that invisible endometriosis, PID or other pathologic conditions are involved in a toxic PF environment.

Heat-inactivation of PF improved fertilitzation and reduced the toxicity or inhibition of gamete interaction when the mouse in vitro fertilitzation assay was performed(Sueldo et al. 1987). In contrast, heat-inactivation and filtered PFs of patients with and without endometriosis maintained irreversible toxicity to mouse embryo development(Morcos et al. 1985). The toxicity of PF from patients with endometriosis is still controversial. In our study, heat-inactiviation of PF did not improve sperm motility and did not reduce toxicity.

Intrauterine or intraperitoneal artificial insemination, in comparison with intracervical, may increase the risk of introducing microorganisms into the peritoneal cavity by bypassing the cervical mucus barrier. However, reports of pregnancy rates after a trial of DIPI have been remarkable. Variable pregnancy rates of DIPI, ranging from 5% to 32.8% per patient, have been reported(Studd et al. 1987;Fenkins & O' Donovan, 1988;Placido et al. 1988). In addition, peritoneal oocytesperm transfer(POST) has been suggested(Mason et al. 1987). Gametes would be exposed to the PF environment in these procedures. Therefore, our study supports the concept that a sperm incubation test with cul-de-sac fluid provides a useful diagnostic index to predict the prognosis of DIPI and POST and offers an appropriated treatment modality. this sperm incubation test is easier to perform, simple, less expensive, and less time-consuming than the mo-

use embryo culture system. Because numerous sperm are used for this test, interpretation is easier and may be more statistically reliable.

Our data show that PF supernatants from infertile patients with endometriosis stage I and II do not have a detrimental effect on sperm motility, but toxic substances(heat-stable) from PFs of some patients not necessarily related to endometriosis may impede sperm motility.

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