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Effect of Peritoneal Fluid from Endometriosis Patients on Sperm Motion Characteristics and Acrosome Reaction

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ABSTRACT: Objective-To determine whether peritoneal fluid from women with endometriosis contributes to infertility by impairing sperm motion and functional characteristics. **Methods-**Women with endometriosis (n = 20) underwent laparoscopy for infertility or pelvic pain. Patients undergoing tubal ligation served as controls (n = 14). Peritoneal fluid was aspirated from women with endometriosis, or from women undergoing laparoscopic tubal ligation. Sperm motility, motion characteristics and acrosome reaction were assessed following incubation with peritoneal fluid. **Results-**Sperm motility, motion characteristics, and acrosome reaction did not differ significantly between the two groups after 3, 5, or 24 hours of incubation with peritoneal fluid. **Conclusions-**Sperm motion or functional characteristics showed no significant impairment when sperm from normal donors were incubated with peritoneal fluid from patients with endometriosis. It is unlikely that peritoneal fluid in these patients contributes to infertility. Int J Fertil 44 (1):31-37, 1999

KEY WORDS: peritoneal fluid, endometriosis, sperm, motility, acrosome reaction

INTRODUCTION

Endometriosis is one of the most common gynecological disorders among women of reproductive age. Although endometriosis can frequently be observed among fertile women, 30% to 50% of women with endometriosis are infertile [1]. Endometriosis may compromise fertility through several mechanisms [2]. The alterations of the anatomical conditions of the pelvis resulting from endometriosis (adhesions, endometriomas) may interfere mechanically with the sperm-oocyte interaction. Another mechanism is based on the molecular pathophysiology of endometriosis. While the effects of cytokines (interleukin-1, tumor necrosis factor-α, interferon-γ, activated macrophages) and macrophages have been investigated in an attempt to identify the constituents of the peritoneal fluid that may be responsible for the inhibition of sperm motility [2,3] and sperm-oocyte interaction [4,5], a direct effect of peritoneal fluid on impairment of sperm motility has not been documented.

There is no consensus whether the peritoneal fluid (PF) is a marker or a causal agent in reduced fertility. Because sperm motility is the best predictor of fertilizing capacity, various studies have addressed the effect of PF on sperm motility. Whereas sperm motility was reported to decrease in infertile women with endometriosis [6-8], others found no changes [9,10]. These differences in results were reported to be due to the preparation of the sperm and PF, variations in the incubation time, and the methods of sperm motility assessment.

The fertilizing capacity of spermatozoa depends on factors such as sperm motility, motion characteristics, and acrosome reaction. The acrosome reaction is a prerequisite for fertilization, but reports about the effect of PF from endometriosis patients on the acrosome reaction are conflicting [9,11,12]. Similarly, decreased sperm binding to the zona pellucida and decreased scores on the hamster-egg penetration assay have been reported when sperm samples were incubated in the presence of PF-containing macrophages [5,13].

Because of the importance of sperm motility and sperm-function (ability to undergo the acrosome reaction), the goal of this study was to assess, objectively, the time-related effects of PF from women with endometriosis on sperm motion characteristics and the extent of the acrosome reaction in the presence of PF.

MATERIALS AND METHODS

Peritoneal fluid samples were obtained from women undergoing diagnostic laparoscopy. To ensure uniformity all women recruited for this study were in the follicular phase of the menstrual cycle. Informed consent was obtained and the study was approved by the Institutional Review Board. Findings at the time of diagnostic laparoscopy were evaluated based on the presence of tubal patency, endometriosis, pelvic adhesions, and pelvic inflammatory disease. The severity of the disease was staged by the revised American Society for Reproductive Medicine classification [14].

Selection of Patients

Twenty endometriosis patients seeking treatment for infertility were grouped into stage 1 (n = 8), stage 2 (n = 7), stage 3 (n = 3), or stage 4 (n = 2). None of these patients with endometriosis had tubal blockage. Control PF was collected from 14 fertile women undergoing tubal ligation for sterilization. None of these women had endometriosis at the time of laparoscopy. All patients with a PF volume of more than 0.5 mL were included in the study. Laparoscopy was performed with a diagnostic laparoscope and an ancillary probe. PF was aspirated from the anterior and posterior cul-de-sac. The specimens were transported to the laboratory and processed immediately in a blinded fashion.

Preparation of Peritoneal Fluid

Since PF of normal women contains approximately 1 x 10^6 cells/mL, and the cellular components are known to affect accuracy of measurement of sperm characteristics, the PF specimens were used after the cellular component was removed. For this, aspirated peritoneal fluid specimens were centrifuged at 500 g for 7 minutes and stored at -20°C. To ensure that no cellular components was present in the PF, the supernatant was filtered through a 0.22-μm sterile Millipore filter (Gelman Sciences, Ann Arbor, MI) and transferred to sterile cryopreservation vials (Corning 1.2 mL cryovials, Fisher Scientific, Pittsburgh, PA) before being added to the sperm samples.

Peritoneal Fluid and Sperm Characteristics

To evaluate the effect of PF on spermatozoa, semen samples were obtained from an established fertile donor, according to World Health Organization criteria [16]. Liquefied semen from a single healthy fertile donor (who had initiated pregnancy in the last year) was used throughout the study. This was done to minimize variations in semen quality because of multiple donor specimens. The semen sample was obtained by masturbation into a sterile specimen jar after an, abstinence of 48 to 72 hours. The ejaculate was allowed to liquefy at 37°C for 30 minutes and washed twice in Biggers-Whitten-Whittingham medium containing 0.3 % human serum albumin (BWW medium; Irvine Scientific, Santa Ana, CA). The sperm pellet was resuspended in approximately 600 μL of the BWW medium. The specimen was processed by the swim-up method to obtain a highly motile population of spermatozoa [17]. In brief, two to three round-bottom tubes (12 x 75 mm polystyrene tubes; Falcon # 2058, Fisher Scientific) were placed at an angle of 45° and 700 μL of the BWW buffer was layered. Two hundred microliters of the sperm pellet was then carefully placed at the bottom of the medium and the specimen tubes were incubated at 37°C in 5% carbon dioxide in air for 60 minutes. The supernatant from each tube was carefully aspirated, pooled and then centrifuged at 300 g for 7 minutes.

To study the effect of PF on sperm motility and motion characteristics, 10 μL of the sperm suspension was added to 100 μL of the PF from endometriosis or tubal ligation patients and incubated at 37°C in 5% carbon dioxide. Controls contained an equal amount of BWW medium instead of the peritoneal fluid. A 5-μL aliquot of the specimen was loaded on a counting chamber (Microcell slide; Conception Technologies, Santa Ana, CA). The slide was analyzed in a blinded fashion on a computer-assisted semen analyzer (CASA, Motion Analysis Corporation, Model VP 110, Santa Rosa, CA) after 0, 3, 5, and 24 hours of incubation. To verify the accuracy of CASA results, samples were also assessed manually for variations in sperm motility and concentration. Sperm motion characteristics such as motility, curvilinear velocity, straight line velocity, average path velocity, linearity and amplitude of lateral head displacement were assessed by CASA.

Peritoneal Fluid and Acrosome Reaction

The acrosome reaction was the functional test examined to study the effect of PF on the spermatozoa from controls and patients with endometriosis. The acrosome reaction was determined using a monoclonal antibody kit (Fertility Technologies, Natick, MA). Immunobeads coated with MH-61 monoclonal antibodies were prepared according to our earlier protocol [17]. A 96-well tissue culture plate was used for the assay. One hundred microliters of PF was added to each well. The sperm concentration was adjusted in each well to give a final concentration of 4.0 x 10^6/mL in the first well, 2.0 x 10^6/mL in the second well, 1.0 x 10^6/mL in the third well, and 0.5 x 10^6/mL in the fourth well. A 10-μL MH-61 bead suspension (1.5 x 10^6/mL) was added to

each well. The contents of the wells were gently mixed, and the culture plate was incubated at 37°C in 5% carbon dioxide. After 0, 3, 5, and 24 hours of incubation (capacitation period), the bead-sperm attachment was observed under an inverted phase-contrast microscope (x 200 magnification), and five fields were counted in each well. The results of all five fields were combined to calculate the percentage of beads bound to sperm, divided by the total number of beads counted.

Statistical Analysis

Wilcoxon's rank-sum test was used to compare the motion characteristics and percentage of Acrobead binding between the endometriosis and control groups. A P value of <0.05 was considered statistically significant. The SigmaStat statistical software package (Jandel Corporation, San Rafael, CA, 1992) was used to analyze the data.

RESULTS

The mean age of patients with endometriosis was 33 years (range: 23 to 41 years). Women undergoing laparoscopic tubal ligation had a mean age of 35 years (range: 29 to 42 years).

The sperm characteristics for the donor sperm used in the entire study were in the normal range described in the WHO guidelines [16]. The specimen had the following characteristics: percentage motility, >50%; concentration, >30 x 10^6/mL; curvilinear velocity, >39 µm/sec; linearity, >19%; average path velocity, >35 µm/sec; amplitude of lateral head displacement, >2 µm. Similarly, after swim-up, a significant improvement in straight line velocity, curvilinear velocity, average path velocity, and amplitude of lateral head displacement was seen. With increasing time of incubation, sperm motion characteristics decreased in specimens incubated with PF from both endometriosis and tubal ligation patients. However, in the endometriosis group, these characteristics showed no, significant difference from those in the tubal ligation group (Table I). When patients with endometriosis were grouped on the basis of stage of endometriosis, although the number of specimens in each group was limited, the stage of endometriosis did not significantly correlate with sperm motility (r = -.07; P <.85). Sperm motion characteristics such as VCL (r =.54; P <.13), VSL (r =.55; P <.12), VAP (r =.54; P <19), and ALH (r =.52; P <.15) also showed no differences. Similarly, in the infertile endometriosis group, sperm motility and motion characteristics showed no significant differences when compared to the fertile endometriosis group.

The Acrobead test was negative in all specimens at 0 hours. The percent binding of sperm to the beads increased with the increase in capacitation. Acrobead binding increased from a median value of 35.5% and 20.0% after 3 hours of incubation to 87.5 % and 79.5 % after 24 hours in endometriosis and tubal ligation groups, respectively. Acrobead binding did not differ significantly between the two groups at 3, 5, or 24 hours of incubation (Table II). The acrosome reaction in the PF from infertile endometriosis patients (n = 15) did not differ significantly from that in fertile endometriosis patients (n = 5) at 3 hours (P = 0.32), 5 hours (P = 0.66), or 24 hours (P = 0.26) of incubation.

TABLE I
Sperm motility and motion characteristics after incubation in peritoneal fluid from women with endometriosis or normal women undergoing tubal ligation.

	Duration of Incubation					
	3 hours		5 hours		24 hours	
Motion Characteristics	Endometriosis	Tubal ligation	Endometriosis	Tubal ligation	Endometriosis	Tubal ligation
motility (%)	84.0 * (81.5, 86.3)	83.5 (66.7, 88.2)	81 (76.5, 84.0)	82.8 (57.0, 90.3)	50 (35.0, 61.3)	50.7 (40.0, 65.7)
Curvilinear velocity (µm/s)	86.6 (78.7, 91.7)	82.7 (64.4, 98.3)	82.5 (77.0, 88.1)	89.1 (64.7, 95.1)	38.8 (19.6, 48.7)	41.1 (37.0, 45.4)
Straight line velocity (µm/s)	-31.7 (27:1, 36.3)	35.2 (23.7, 37.3)	35.4 (33.1, 38.6)	36.9 (22.3, 40.6)	14.1 (6.7, 18.4)	15.7 (14.4, 18.5)
Average path velocity (µm/s)	56.5 (51.3, 61.6)	55.3 (44.8, 67.9)	54.3 (52.4, 61.6)	58.9 (43.5, 65.8)	22.1 (11.7, 32.6)	27.5 (24.1, 29.8)
Linearity (%)	35.8 (35.2, 41.7)	38.8 (37.9, 42.4)	40.4 (37.8, 43.1)	38.6 (35.0, 42.3)	34.7 (30.1, 38.5)	37.5 (31.5, 39.1)
Amplitude of lateral head displacement (µm)	2.5 (2.3, 2.7)	2.4 (1.9, 2.8)	2.4 (2.3, 2.6)	2.4 (1.9, 2.6)	1.3 (0.9, 1.5)	1.4 1(1.2, 1.5)

*Median (25th, 75th percentiles)

TABLE II
Sperm acrosome reaction after incubation in peritoneal fluid from women with endometriosis or normal women undergoing tubal ligation.

Incubation Time	Percent Acrobead Binding		
(hours)	Endometriosis	Tubal ligation	P*
3	35.5 (19.5, 50.0)†	20.0 (9.0, 48.0)	0.37
5	42.0 (26.0, 55.5)	24.5 (11.0, 66.0)	0.53
24	87.5 (59.5, 96.0)	79.5 (50.0, 91.0)	0.4

*Wilcoxon rank-sum test
†Median (25th, 75th percentiles)

DISCUSSION

The pathophysiologic mechanism of endometriosis and endometriosis-related infertility is unclear [18]. Studies have demonstrated the presence of fertility inhibiting factor(s) located in the cell-free fraction; this factor is predominant in the PF from women with endometriosis [19,20]. We used a cell-free fraction of the PF in our study, and the sperm samples were prepared by the swim-up technique to remove the seminal plasma, thus more closely simulating the sperm environment in the upper female genital tract.

Reports on the in vitro and in vivo effects of PF from endometriosis patients on sperm motility are contradictory [6-7,10,20-22]. Conflicting results may be the result of lack of an ideal control in the studies. Some researchers used fertile or infertile patients without endometriosis as controls [6,10,20,21], whereas others used only fertile patients without endometriosis [7,22]. Even fertile patients without endometriosis as confirmed by laparoscopy may have endocrine disorders, ovarian disease, pelvic pain, and or other disorders. Women with multiple parity undergoing laparoscopic sterilization, a nearly ideal control group, were used in our study.

Sperm motility and motion characteristics did not differ between the endometriosis and tubal ligation groups at any of the data collection times, indicating that the PF does not directly inhibit sperm motility or motion characteristics in endometriosis patients. Similar results were seen when PF of women with minimal or mild endometriosis was incubated over a period of 24 hours [22]. However, a decrease in the various sperm motion characteristics was seen when PF fluid from patients with moderate or severe endometriosis was used [22]. Furthermore, these differences disappeared when Percoll-purified semen specimens were used. Some studies have used unwashed semen samples in the assessment of PF-sperm interaction and concluded that PF from endometriosis has an adverse effect on sperm motion characteristics [6,7]. Interaction between factors within the seminal plasma and PF may contribute to the diverse effects observed [3,7]. In our study, we used normozoospermic samples from a healthy fertile donor and prepared sperm by the swim-up technique to remove proteolytic enzymes present in, the seminal plasma. We also employed CASA, which allows for more objective and more detailed analysis of sperm movement. We did not use the whole (unfiltered) PF to study the effect on sperm motion or the acrosome reaction because of the presence of obvious cellular contamination. It is quite likely that unfiltered PF may influence the sperm motion and functional characteristics. Similarly, during filtration, the factor(s) responsible for inhibition may bind to the filter and be excluded. Furthermore, differences exist in the male population such that certain donors may be more susceptible to the effects of endometriotic PE Therefore, we performed the entire study with semen specimens obtained from a single donor of proven fertility to rule out semen variations. These could explain the differences between our results and some of the earlier studies.

The reported effects of PF from endometriosis patients on sperm acrosome reaction are also conflicting [9,11,12]. These conflicting results may be due to improper controls. The Acrobead test, which uses a monoclonal antisperm antibody test, was developed recently to assess the acrosome reaction. The results of the acrosome assay correlate highly with in vitro fertilization outcome [23,24].

The sample size of patients with endometriosis was too small to provide a meaningful interpretation based on stage of the disease; therefore, we studied the overall effect of the PF from patients with endometriosis. Fourteen normal fertile women undergoing laparoscopic tubal ligation served as controls. Different ratios of PF to sperm suspension were used by other investigators to study the effect of PF on the acrosome reaction. The ratio of PF to sperm suspension (vol/vol) ranged from 2:1 to 5:1 in some studies [11,12]. A PF to sperm suspension ratio of 10:1 was used in our study to simulate the physiological environment. The Acrobead binding in endometriosis cases did not differ from that from women undergoing laparoscopic tubal ligation, indicating that PF from endometriosis patients did not adversely affect the sperm acrosome reaction. Again, in our study, the PF in endometriosis patients did not inhibit sperm motion characteristics or acrosome reaction. Our study also supports the contention of previous studies that PF from endometriosis patients does not adversely affect sperm acrosome loss [9].

Infertility can result from the altered peritoneal-fluid environment in patients with minimal or mild endometriosis [12,25]. PF proteins obtained from women with moderate to severe endometriosis are significantly increased in the luteal phase compared with those obtained from mild endometriosis and normal controls. This could contribute to the conflicting results reported by various authors. We found no correlation between the stage of endometriosis and sperm characteristics when fertile and infertile groups of women with endometriosis were compared. It must, however, be mentioned here that the sample size for each stage of endometriosis was a limiting factor. PF from patients, irrespective of the stage of endometriosis, did not affect sperm motion characteristics after 24 hours [26]. A recent study has shown that laparoscopic resection or-ablation of minimal and mild endometriosis enhances fecundity in infertile women [27]. Similar IVF pregnancy rates were seen in patients diagnosed with either endometriosis or tubal disease [28,29]. On the contrary, higher pregnancy rates per cycle and per transfer were observed in advanced-stage endometriosis [30], suggesting an anatomical distortion of the pelvis found in the advanced stage which is bypassed during IVF. The lower implantation rate reported in patients with endometriosis compared with other groups of patients may be due to an impaired relationship between the embryo and the endometriosis: abnormal implantation may be secondary to endometrial dysfunction, or the embryonal environment may be a factor in endometriosis -associated subfertility [30]. In an elaborate study of this by the Norfolk group, no differences in pregnancy or fertilization rates were seen [31]. The PF might cause infertility in endometriosis patients by affecting sperm phagocytosis, sperm-oocyte interaction, ovum capture by oviduct fimbriae, early embryo development, and implantation [3].

Again, conflicting reports may be due to lack of an ideal control group. Most in vitro fertilization studies have failed to document an effect of endometriosis on fertilization and pregnancy rates, indicating that the presence of endometriosis does not influence oocyte quality or success of fertilization in in vitro fertilization [25,32]. Similar pregnancy outcome has been observed in patients with endometriosis compared to patients with mechanical (tubal infertility) [33], and no differences were seen in pregnancy rates by stage of endometriosis. Abnormal implantation may be secondary to endometrial dysfunction, or a toxic embryonal environment may be a factor in endometriosis-associated subfertility. Thus, despite normal sperm motility and acrosome reaction, there could be failure of sperm binding, egg penetration, and eventual fertilization in patients undergoing IVF. Therefore, a lack of observed in vitro effect does not prove an absence of effect on sperm function. It is quite possible that other fertilization mechanisms, such as zona or vitelline membrane binding, may also be affected.

In conclusion, with our limited sample size, we were unable to demonstrate a difference in sperm motion or functional characteristics following incubation with peritoneal fluid from women with endometriosis. It is unlikely that peritoneal fluid in these patients contributed to their infertility.

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