Endometriosis is common in women with infertility, but the etiology of infertility in these women is unclear. The adhesion and concomitant distortion of the pelvic anatomy may account for the infertility in advanced endometriotic patients. However, infertility is difficult to explain in patients with minimal or mild endometriosis. There are conflicting reports in the literature that peritoneal fluid (PF) of patients with endometriosis could adversely affect the spermatozoal motion characteristics. The aim of the study was to 1) determine the effect of PF on sperm motion parameters, and 2) assess its effect on sperm acrosome reaction (AR). PF was aspirated from endometriotic and tubal ligation patients (serving as controls) during laparoscopy. Sperm from normal healthy men were incubated for 3, 5, and 24 hours with PF from endometriotic (n = 13) and tubal ligation (n = 14) patients to assess sperm motion parameters by a computer-assisted semen analyzer. Sperm AR was evaluated by a monoclonal antibody kit “Acrobead test” in endometriotic (n = 20) and tubal ligation (n = 14) patients. No significant differences were seen in sperm motion parameters and percentage of AR between endometriotic and tubal ligation patients. These results suggest that PF from patients with endometriosis may not directly affect the sperm function. It could have a deleterious effect on the egg, sperm-oocyte interaction or early embryo development.