The impact of peritoneal fluid from healthy women and women with endometriosis on sperm DNA and its relationship to the sperm deformity index

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Introduction: The sperm deformity index (SDI) is a novel method of assessment of the sperm morphology. SDI is a powerful predictor of the fertility potential of semen samples. Sperm deformity score >1.6 has been shown to be linked to sperm chromosomal abnormalities. The relationship between endometriosis and infertility is not completely understood. The impact of peritoneal fluid from patients with endometriosis on sperm chromatin integrity is a potential cause of infertility in these cases. This study aimed to assess the impact of peritoneal fluid (PF) from healthy women and women with endometriosis on sperm DNA and its relationship to sperm morphology as assessed by the sperm deformity index (SDI).

Material & Methods: Sperm aliquots prepared from 10 healthy donors were incubated with peritoneal fluid (PF) from healthy women undergoing laparoscopic tubal ligation (Treatment 1, n = 10), PF from patients with endometriosis (Treatment 2, n = 10) and HTF with 10% BSA (Control, n = 10). Sperm DNA fragmentation was assessed by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay after 1.5, 3 and 24 hours incubation in all three experiments. The samples were analyzed using a FACScan flow cytometer (BD Bioscience, San Jose, CA) with an air-cooled argon laser operated at 488 nm and a power of 15 mW. Sperm morphology and the sperm deformity index scores were assessed using the strict criteria. Seminal smears were stained with Diff-Quik stain (Diff-Quik, Baxter Scientific Products, McGaw Park, USA). The SDI was calculated by dividing the total number of deformities observed by the number of sperm randomly selected and evaluated, irrespective of their morphological normality. Data were analyzed using inbuilt functions within the Statistical Package for Social Science (SPSS UK Ltd., Chertsey, Surrey, UK). The analysis of variance was utilized to compare
mean sperm DNA damage for different treatments and to compare mean sperm DNA damage for different incubation interval for each treatment. Spearman's rank correlation was utilized to provide a test of independence between sperm DNA damage and sperm morphological features. All hypothesis testing was two-tailed and $P < 0.05$ was considered statistically significant.

**Results:** There was significant increase in sperm DNA damage at 24 h compared to 1.5 and 3 h ($p<0.01$). At each studied time interval there was significantly higher sperm DNA damage in aliquots incubated with endometriosis PF compared to healthy women PF and to HTF control. The mean SDI score was $1.74 \pm 0.19$. There was evidence of significant positive interdependence between the SDI scores and sperm DNA damage in all aliquots incubated with endometriosis PF [At 1.5 h: $r = 0.87$ ($P = 0.002$); at 3 h: $r = 0.81$ ($P = 0.0068$); at 24 h: $r = 0.85$ ($P = 0.003$)]. There was also evidence of significant positive interdependence between the SDI scores and sperm DNA damage in treatment 1 and control samples incubated for 1.5 and 24 h (data will be presented).

**Conclusions:** In vitro exposure of sperm to PF from endometriosis patients is associated with significantly increased DNA damage compared to healthy women. In these patients the significant increase in sperm DNA damage observed after 24 h incubation may negatively impact their natural fertility and may compromise the outcome of assisted reproduction techniques. There is evidence of interdependence between the sperm morphology as assessed by SDI scores and DNA damage.