Objective: NFκB (p65, p50 and IκB) plays a major role in regulating apoptosis. Its role in the pathogenesis of male infertility has never been studied. The objectives of our study were to examine 1) the relationship of NFκB with semen quality (sperm concentration, motility and morphology) and the clinical diagnoses of male infertility, and 2) correlate levels of NFκB and apoptosis in ejaculated human spermatozoa.

Design: Prospective experimental design

Materials/Methods: Semen samples from 12 donors and 40 infertile men (varicocele, n = 13; and others, n = 27) were used to probe for levels of p65, p50 and IκB molecules. Semen samples were stratified into patients with poor sperm concentration (<20 X 10^6/mL; n = 11) and patients with normal sperm concentration (>20 X 10^6/mL; n = 29). Levels of apoptosis were detected using Annexin-V staining in neat semen specimens. The assay detects externalization of phosphatidylserine to the outer surface of the plasma membrane of apoptotic spermatozoa. The percent apoptosis was determined by epifluorescent microscopy. Western Blot technique was used to analyze for p65, p50 and IκB proteins using rabbit polyclonal anti p65, p50 and IκB antibodies. After treating with secondary antibody conjugated to horseradish peroxidase, antigen-antibody reaction was visualized by an enhanced chemiluminescence assay. Bands were quantitated using the Alpha Fluorochem analyzer and Actin counter staining was done on the blots to ensure that equal amounts of protein were loaded.

Results: Using univariate regression analyses, sperm concentration was directly correlated with levels of p65 (P = 0.01), p50 (P = 0.05), and IκB (P = 0.01). Patients with poor sperm concentration had significantly lower levels of NFκB as compared to patients with normal sperm concentration. Infertile men had significantly higher levels of apoptosis (p = 0.02) and lower levels of NFκB (p <0.001) as compared to normal donors. Levels of p50 was significantly lower in varicocele patients when compared to patients with no clinical varicocele (p = 0.03).

Conclusions: These results demonstrate significant correlation between low sperm concentration and low levels of NFκB expression. We speculate that low expression of NFκB is related at least in part to poor sperm production in infertile men.

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