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Deterioration of Plasma Membrane Is Associated With Activated Caspases in Human Spermatozoa

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Spermatozoa with deteriorated plasma membranes can be separated by magnetic-activated cell sorting (MACS) after binding superparamagnetic annexin V-conjugated microbeads (ANMBs) to membrane phosphatidylserine (PS).

Semen samples from 15 donors and 25 infertile patients were divided into 2

spermatozoal fractions by annexin V-MACS. Activated caspases (aCPs), which mediate degradations of cell quality, were determined by CaspaTag in the 2 subpopulations. Spermatozoa from donors showed lower levels of bound annexin V ($3.6\% \pm 0.5\%$ vs $11.9\% \pm 1.1\%$; $P < .01$) and aCPs ($21.8\% \pm 2.6\%$ vs $43.2\% \pm 2.1\%$; $P < .01$) than did spermatozoa from infertile patients. MACS resulted in a decrease of spermatozoa with aCPs from $21.8\% \pm 2.6\%$ (before separation) to $9.2\% \pm 1.4\%$ (in the ANMB-negative fraction) in donors and from $43.2\% \pm 2.1\%$ to $18.8\% \pm 2.6\%$ in infertile patients (mean \pm SEM; $P < .01$). Separation effects of the MACS technique were confirmed with flow cytometry using anti-annexin V antibodies and with electron microscopy. ANMB-MACS removes spermatozoa with PS-bound annexin V and produces a higher quality spermatozoal fraction. Spermatozoa with a deteriorated membrane are characterized by an increase in aCPs. A higher percentage of spermatozoa with ANMBs bound to PS and with aCPs were found in infertile patients.

Key words: Annexin V, immunomagnetic cell depletion, human spermatozoa, caspases, electron microscopy

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