BestSpermSelect - A Novel Technique of Diagnostic and Therapeutic Human and Animal Sperm Selection Based on Early Apoptotic Markers

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Technology description

Method to improve selection of sperm for use in In Vitro Fertilization by detecting and removing sperm that look healthy but are damaged, leaving only truly healthy sperm.

Sperm chromatin damage may be a negative predictor for IVF outcomes in couples with recurrent spontaneous abortions and poor embryo development. Unfortunately, available methods for detecting chromatin damage such as SCSA, TUNEL, Halosperm and Comet assays require permanent fixation of sperm, which render the tested sperm unsuitable for clinical use.

Early apoptotic events resulting in chromatin damage are associated with increased permeability of the cell membrane to large ions. We are developing the use of a large fluorescent organic cation (PF-1) for fluorescence-activated cell sorting to select sperm without chromatin damage.

Proof of concept

Semen samples from 18 men were analyzed using propidium iodide (PI) and proprietary fluorochrome (PF-1). Dead sperm are permeable to PI, and apoptotic cells are permeable to PF-1. Intact cells are impermeable to both. Hoechst 33342 was used to calibrate the BD LSR II flow cytometer. Semen samples from five patients were sorted with the BD FACSVantage. Normal spermatozoa were separated from necrotic and apoptotic cells based on fluorochrome staining (See fgure below). Percoll density gradient was used to remove debris prior to sorting. To verify selection of intact sperm, the stained and unstained populations were examined microscopically for motility and viability, and analyzed for DNA fragmentation with the TUNEL assay. The two populations were compared using the chi-square test for difference in the percentage of TUNEL-positive cells.

In the group positive for PF-1 and PI, 431 of 2,167 sperm (19.5 %) were TUNEL positive. In the non-staining group only 33 of 2,263 (1.5%) were TUNEL positive. The difference was highly statistically significant ($p<0.00001$). Unstained sperm had excellent progressive motility and normal morphology.

This technology allows for sperm chromatin damage analysis as well as quick and reliable sorting, separating normal sperm from those with chromatin damage. Because the test employs large molecules that require activation of sodium channels to enter sperm, there is little risk for residual fluorochrome in the isolated specimen. This assay may be a new treatment modality for couples with male factor infertility secondary to sperm chromatin damage.

Institution

Cornell University

Inventors

Darius Paduch
Peter Schlegel