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Contact/Presenting Author: Nabil Aziz, MD

Department/Institution: Reproductive Research Center, Cleveland Clinic

Address: 9500 Euclid Avenue A19.1

City/State/Zip/Country: Cleveland, Ohio, 44195

Phone: 1-216-444-4402 **Cell Phone:** **Fax:** 1-216-445-6049 **E-mail:**
agarwaa@ccf.org

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Title: Association of sperm morphology assessed by sperm deformity index (SDI) with Poly (ADP-Ribose) polymerase (PARP) cleavage inhibition

Nabil Aziz, MD, agarwaa@ccf.org, Reda Mahfouz, MD, mahfour@ccf.org, Rajesh Jha, Ph.D, jhar@ccf.org, Rakesh Sharma, Ph.D, sharmar@ccf.org, Maria Bykova, Dipl.Specialist, bykovam@ccf.org and Ashok Agarwal, Ph.D, HCLD, agarwaa@ccf.org. ¹Reproductive Research Center, Glickman Urological Institute and Department of Obstetrics & Gynecology, Cleveland Clinic, Cleveland, Ohio, United States, 44195 and ²Department Of Obs/Gyn, Liverpool Women's Hospital, Liverpool, United Kingdom, L87SS.

Objective: The sperm deformity index (SDI) is a novel expression of sperm morphology that has been shown to be a powerful predictor of the fertility potential of a semen sample both in vivo and in vitro. We have previously demonstrated a positive correlation between the SDI and late markers of sperm apoptosis. Cleavage of Poly (ADP-Ribose) polymerase (cPARP) has been shown to be stimulated by any DNA damage and so it is considered to be an early apoptosis marker. The association of cPARP with sperm morphology as assessed by the SDI has not been studied before. The aim of our study was to evaluate the relationship between the SDI and cPARP.

Design: Prospective study.

Materials and Methods: Semen samples were collected from 10 donors and prepared by sperm density gradient separation. The resultant mature and immature sperm fractions were examined for sperm motility applying the WHO 1999 standards. Sperm morphology was assessed using the strict criteria of morphology. A multiple entry technique was utilized to calculate the SDI scores. Two aliquots of each of the mature and the immature fraction were used to induce sperm apoptosis by adding staurosporine 2.5uM final concentration, with and without PARP inhibitor (3-aminobenzoate; 3-ABA, final concentration 0.3 mM). Aliquots were examined for cPARP using flow cytometry. Paired Mann-Whitney test was utilized to compare different variables in different sperm fractions. Spearman's rank correlation was used to examine relationships between variables. $P < 0.05$ was considered significant.

Results: The mature sperm fractions had significantly lower mean SDI score and significantly higher mean percentage sperm with normal morphology compared to the immature fractions (normal morphology%: CI = 5 to 10, $P < 0.0001$; SDI: CI = -0.39 to -0.14, $P = 0.0006$). When the data was considered collectively there was a significant positive interdependence between the SDI scores and cPARP positive sperm in the treated sperm with PARP inhibitor ($r = 0.5$, $P = 0.039$). On the other hand, there was no correlation between percentage sperm with normal morphology and cPARP positive sperm in the treated sperm with PARP inhibitor ($r = -0.02$, $P = 0.9518$).

Conclusions: The sperm deformity index scores are associated with PARP cleavage as an early marker of apoptosis. PARP inhibition in sperm samples with high SDI score promotes induced apoptosis reflecting an inbuilt susceptibility to apoptotic

stimuli.

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