Evaluation of poly(ADP-ribose) polymerase cleavage (cPARP) in ejaculated human sperm fractions after induction of apoptosis

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Objective: To examine the presence of cleaved poly(ADP-ribose) polymerase(s) (cPARP) in ejaculated spermatozoa and determine cPARP levels following exposure to chemical or oxidative stress.

Design: Prospective pilot study.

Setting: Tertiary care academic hospital.

Patient(s): Eight healthy men.

Intervention(s): Semen specimens were collected, prepared with double-density-gradient centrifugation, and divided into control, hydrogen peroxide (H₂O₂), H₂O₂ + 3-aminobenzamide (3-ABA), staurosporine (STS), and STS + 3-ABA treated groups.

Main Outcome Measure(s): Cleaved PARP and apoptosis markers by flow cytometry.

Result(s): Cleaved PARP was detected in both neat and mature fractions. The cPARP levels were similar in both mature and immature spermatozoa. In combined mature and immature fractions, a higher percentage of late apoptotic sperm was seen in STS + 3-ABA versus STS. Higher levels of late apoptotic spermatozoa were seen in immature versus mature fractions within STS and STS + 3-ABA groups. Lower levels of cPARP were seen in immature versus mature fractions in H₂O₂ and H₂O₂ + 3-ABA treated groups. Cleaved PARP was related to activated caspase-3.

Conclusion(s): Cleaved PARP is present in ejaculated human spermatozoa. Poly(ADP-ribose) polymerase inhibitors may play a different role in chemical versus oxidative stress-induced sperm damage. (Fertil Steril 2009;91:2210–20. ©2009 by American Society for Reproductive Medicine.)

Key Words: Apoptosis, flow cytometry, PARP cleavage, sperm fraction, TUNEL

Male factor infertility is solely responsible for about 20% of infertility cases and is contributory in another 30 to 40% (1, 2). The possible etiologies of male infertility include idiopathic causes, varicocele, extensive iatrogenic interventions, and erectile dysfunction as well as defective spermatozoa (3–7). One possible cause of male infertility that has been studied intensely in the last decade is the integrity of DNA in the nucleus of mature ejaculated spermatozoa (8, 9). There are many possible causes of sperm DNA damage, including abortive apoptosis, oxidative stress associated with infections, chemical exposure, and defects of spermiogenesis associated with the retention of excess residual cytoplasm (10–16). The focus on the genomic integrity of the male gamete has been intensified by the growing concern about transmission of genetic diseases through intracytoplasmic sperm injection (ICSI) (17).

Apoptosis is defined as programmed cell death, which is an energy- and protein-dependent process which depends on the cellular adenosine triphosphate (ATP) level (18). Human spermatozoa have the ability to undergo apoptosis or apoptosis-like conditions (19–27). Absence of Fas protein from the spermatozoa or dysfunction has been reported (28, 29), and another study reported presence of Fas ligand on the mature ejaculated spermatozoa (30–32). Sperm DNA damage can occur at the time of, or be the result of, DNA packaging during spermiogenesis stages through abortive apoptosis (33–37).
Poly(ADP-ribosyl)ation is a DNA damage–dependent process which is affected by cell energy level. Poly(ADP-ribose) (PAR) level is controlled by the opposing actions of poly(ADP-ribose) polymerase (PARP) and poly(ADP-ribose) glycohydrolase, a major enzyme responsible for the catabolism of PAR. Poly(ADP-ribose) metabolism is critical in a wide range of biologic structures and processes. These include DNA repair and maintenance of genomic stability, transcriptional regulation, centromere and centrosomal function, mitotic spindle formation, telomere dynamics, trafficking of endosomal vesicles, apoptosis, and necrosis. In humans, the PARP family is comprised of as many as 18 distinct proteins; PARP-1 is the most expressed member. Poly(ADP-ribose) polymerase 1 plays a primary role in the process of poly(ADP-ribose)ylation. It has been implicated in genome maintenance, carcinogenesis, aging, immunity, inflammatory conditions, and neurologic function.

Poly(ADP-ribose) polymerase 1 has a well-characterized role in DNA damage detection and repair. Protein ribosylation by PARP-1 is an immediate response to DNA damage induced by oxidants, alkylation, or ionizing radiation. In contrast to its role as a survival factor in limited DNA damage, PARP-1 promotes cell death under conditions of extensive DNA damage. Therefore, chemical inhibition or genetic ablation of PARP-1 can provide protection against cell death, including ischemia-perfusion injury, cardiac infarction, neural cell damage, or aging.

Poly(ADP-ribose) polymerase has been documented consistently as playing a functional role in testicular germ cells. Germ cells require PARP for repair of DNA damage. Maymon et al. detected PAR expression in germline cells. Its subcellular localization in meiotic and postmeiotic spermatogenesis stages demonstrated chromatin modifications occurring during spermatogenesis. Although spermatids do not enter a prophase, Maymon et al. concluded that PARP expression in germline cells and its subcellular localization in meiotic and postmeiotic prophase demonstrate chromatin modifications occurring during spermatogenesis. Their results showed that PARP was localized in germ cell nuclei in full spermatogenesis (in round and elongated spermatid and primary spermatocytes). This establishes a key role for PAR in germ cell differentiation, presumably to safeguard DNA integrity.

Besides the established central role of PARP-1 and PARP-2 in the maintenance of genomic integrity, accumulating evidence indicates that PAR may modulate epigenetic modifications under physiologic conditions. Poly(ADP-ribose) polymerase 2 exerts essential functions during meiosis I and haploid gamete differentiation. However, a similar role for PARP in human ejaculated spermatozoa is controversial. Whether PARP can repair DNA damage in mature human spermatozoa remains largely unclear. Oocyte repair capacity will help in the presence of sperm DNA damage. However, recently PARP was shown to have a role in full spermatogenesis, and we have reported its presence in different homologues. Poly(ADP-ribose) polymerase may protect the sperm DNA during the maturation/transportation processes. In addition, it may play a role in sperm chromatin remodulation.

Conflicting reports exist regarding the presence of whole or cleaved PARP. Recently we reported the presence of PARP homologues in ejaculated human spermatozoa and identified these as PARP-1 (~75 kDa), PARP-9 (~63 kDa), and PARP-2 (~60 kDa). We demonstrated a positive correlation between the amount of PARP protein and sperm maturity, suggesting a role for PARP proteins in sperm DNA damage/repair.

The goal of the present study was to validate the presence of cPARP in ejaculated human spermatozoa and determine whether the cPARP level can be modulated by chemical or oxidative stress cell injury in the presence or absence of PARP inhibitor.

MATERIALS AND METHODS
Sample Collection
A pilot study was conducted after Institutional Review Board approval. Following 48–72 h of sexual abstinence, semen samples were collected from eight healthy donors, defined as a group of healthy male volunteers with sperm parameters in the normal range (normozoospermic) according to World Health Organization guidelines. These included men with proven and unproven fertility. Sample collection was done at the Cleveland Clinic Andrology Laboratory by masturbarion into sterile containers. The experimental design of the study is shown in Figure 1.

Standard Semen Analysis
After liquefaction, semen specimens were evaluated for volume, sperm concentration, total cell count, motility, and morphology. A 5-μL aliquot of the sample was loaded on a Microcell slide chamber for manual evaluation of concentration and motility.

Separation of Mature and Immature Sperm Population
Liquefied semen (1–2 mL) was loaded onto a 40% and 80% discontinuous Pureception density gradient (Sage Assisted Reproduction Products, Bedminster, NJ) and centrifuged at 1600 rpm (400g) for 20 min at room temperature. The resulting 80% pellet, representing the mature fraction, and 40% cell layer, representing the immature fraction, were separately suspended in human tubal fluid (HTF) media and centrifuged at 300g for an additional 7 min to remove the remaining medium.

The interphase between the 40%–80% gradient comprising the immature sperm was carefully aspirated into a tube, washed, and resuspended in sperm wash medium. This fraction consisted largely of immature morphologically abnormal sperm with poor motility. Similarly, the pellet at the
bottom of the 80% gradient was carefully aspirated into another tube, washed, and resuspended in sperm wash medium. This fraction consisted of morphologically normal, highly motile, mature sperm. Both the mature and immature sperm fractions were washed with Quinn’s sperm wash media (Sage In Vitro Fertilization, San Clemente, CA), and final washing was done with phosphate-buffered saline (PBS, pH 7.4).

**Induction and Modulation of Apoptotic and Oxidative Sperm DNA Damage**

In vitro sperm DNA damage was induced using: 1) staurosporine (STS, 10μmol/L; Sigma-Aldrich, St. Louis, MO) incubation at 37°C for 4 h (56); and 2) hydrogen peroxide (H₂O₂, 100μmol/L; Sigma-Aldrich) incubation at 37°C for 1 h (57). Poly(ADP-ribose) polymerase inhibition was achieved using 3-aminobenzamide (3-ABA, 1 mmol/L; Sigma-Aldrich) (58).

**Assessment of Cleaved Poly(ADP-Ribose) Polymerase**

The presence of cPARP was detected using an FITC-conjugated anti-PARP cleavage site–specific antibody (CSSA) kit (ApoTarget, Anti-PARP CSSA FITC apoptosis detection kit; BioSource International, Camarillo, CA) as described earlier (59). Briefly, sperm pellets were washed twice with PBS by centrifugation at 300g, and the intracellular staining protocol was followed according to the manufacturer’s instructions. Sperm pellets were fixed in IC Fix buffer for 20 min at 4°C at a concentration of 1 mL IC Fix buffer per 1 × 10⁹ sperms. Tubes were spun at 300g for 5 min; the supernatant was discarded, and the pellets were washed twice in PBS. Cells were resuspended in IC Perm buffer to yield a density of 1 × 10⁹/50 μL. Ten microliters of FITC-conjugated anti-PARP CSSA was incubated with the treated and untreated sperm cell suspensions at room temperature for 30 min. Samples were washed twice in 2 mL IC Perm buffer.
and once in PBS and resuspended in 0.5 mL PBS for fluorescence-activated cell sorter (FACS) analysis.

Terminal Deoxynucleotidyl Transferase–Mediated Fluorescein-dUTP Nick-End Labeling Assay
Sperm DNA fragmentation was evaluated using the terminal deoxynucleotidyl transferase–mediated fluorescein-dUTP nick-end labeling (TUNEL) assay kit (Apo-Direct; BD Biosciences Pharmingen, San Diego, CA) as described earlier (60–63). Briefly, 1 × 10⁶ spermatozoa were washed in PBS, resuspended in 1% paraformaldehyde, and placed on ice for 30–60 min. Subsequently, spermatozoa were washed again and resuspended in 70% ice-cold ethanol.

Following a second wash in PBS to remove the ethanol, sperm pellet samples as well as the positive and negative controls provided with the assay kit were resuspended in 50 µL of the staining solution for 60 min at 37°C. The staining solution contained terminal deoxytransferase (TdT) enzyme, TdT reaction buffer, fluorescein-tagged deoxyuridine triphosphate nucleotides (FITC-dUTP), and distilled water. All cells were further washed in rinse buffer, resuspended in 0.5 mL of propidium iodide/RNase solution, and incubated for 30 min in the dark at room temperature followed by flow cytometric analysis.

Annexin V/Propidium Iodide Assay
To perform this assay, the annexin-V FITC Apoptosis Detection Kit was used (BD Biosciences Pharmingen). A 100-µL aliquot of neat, mature, and immature sperm fractions was resuspended in 400 µL cold reaction buffer (HEPES; N2-hydroxyethyl piperazine-N2-ethanesulfonic acid) containing 2.5 mmol/L CaCl₂. Sperm cells were labeled with 10 µL each of annexin-V/FITC solution and propidium iodide (PI) for detecting apoptotic and necrotic sperm. Samples were incubated for 15 min at room temperature in the dark. Cells were washed with 1 mL PBS, centrifuged, and resuspended in 300 µL reaction buffer. Flow cytometric analysis was done to quantitatively determine the percentage of early apoptotic and late apoptotic, necrotic, and viable cells. Early apoptotic sperm were those that were positive for both annexin V and PI (annexin V⁻/PI⁺) and late apoptotic sperm were those that were positive for annexin V (annexin V⁺), and late apoptotic sperm were those that were positive for both annexin V and PI (annexin V⁺/PI⁺) (64–66).

Active Caspase-3 Staining
Assessment of active caspase-3 activity was performed using a phycoerythrin-conjugated monoclonal active caspase-3 antibody apoptosis kit (BD Biosciences Pharmingen) as established earlier (67). Briefly, sperm pellets were washed twice with cold PBS, resuspended in 0.5 mL Cytofix/Cytoperm solution/10⁶ sperm, and incubated with the cytofix for 20 min on ice. Centrifugation was done at 300g; supernatant was aspirated and discarded. Sperm pellets were washed twice with 0.5 mL Perm/Wash buffer/10⁶ sperm. For each test tube, 100 µL Perm/Wash buffer and 20 µL antibody were added and incubated for 30 min at room temperature. Washing was done in 1.0 mL Perm/Wash buffer followed by resuspension in 0.5 mL buffer solution. All samples were analyzed by flow cytometry.

Flow Cytometry Analysis
All fluorescence signals of labeled spermatozoa were analyzed by the flow cytometer FACScan (BD Biosciences). Approximately 10,000 spermatozoa were examined for each assay at a flow rate of <100 cells/s. The excitation wavelength was 488 nm supplied by an argon laser at 15 mW. Green fluorescence (480–530 nm) was measured in the FL-1 channel and red fluorescence (580–630 nm) in the FL-2 channel. Gating was done to exclude debris and aggregates using 90° and forward-angle light scatter. The percentage of positive cells and the mean fluorescence were calculated on a 1,023-channel scale using the flow cytometer software FlowJo version 6.4.2 (Tree Star, Ashland, OR).

Statistical Analysis
Data were represented as mean ± SD or median (25th–75th percentiles). We used the Wilcoxon signed rank test for comparisons between groups and the Spearman nonparametric test for correlations. All statistical analysis was done by using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). A P value of <.05 was considered to be statistically significant.

RESULTS
Ejaculated sperm had a semen volume of 2.85 ± 1.17 mL, concentration 80.2 ± 50.5 × 10⁶/mL, median 65.9 × 10⁶/mL (48.7–136.1 × 10⁶/mL), and motility 64.2 ± 9.15%.

Apoptotic Markers in Mature and Immature Sperm Without PARP Modulation
Detectable levels of cPARP were seen in both mature and immature spermatozoa using the intracellular staining technique followed by detection with flow cytometry (Fig. 2A). The percentage of sperm that were positive for cPARP was similar in both mature and immature sperm (Figs. 3).

PARP Modulation Experiment in the Presence and Absence of Inducer/Inhibitor
Mature and immature sperm were exposed to PARP modulation by chemical injury (STS) or an oxidative stress inducer (H₂O₂) in the presence or absence of the PARP inhibitor 3-aminobenzamide (3-ABA). Results are shown in Tables 1 and 2 and Figures 2 and 3.

PARP Cleavage (cPARP Assay)
The cPARP-FITC fluorescence intensity in the mature fraction was higher in the STS-only group compared with the H₂O₂-only group (Fig. 2B). Higher fluorescence intensity in cPARP⁺ve staining was seen in immature sperm in the STS-only group compared with the H₂O₂-only or control groups (Fig. 2C). Exposure to the PARP inhibitor 3-ABA
decreased the cPARP-FITC fluorescence intensity in the STS-only group compared with the H₂O₂-only groups (Fig. 2D).

**DNA Fragmentation by TUNEL assay**
Immature and mature sperm exposed to STS or H₂O₂ alone or in the presence of 3-ABA did not exhibit a significant difference in the percentage of TUNEL⁺ve sperm compared with the control group (Figs. 3C and 3D).

We next combined the immature and mature sperm groups to examine the effect of 3-ABA on the percentage of TUNEL⁺ve sperm. A higher but nonsignificant percentage of TUNEL⁺ve sperm was seen in both STS + 3-ABA versus STS-only groups and H₂O₂ + 3-ABA-treated versus H₂O₂-only groups (Table 1; Figs. 3C and 3D).

**Apoptosis by Annexin V/PI Assay**

**Early apoptosis** Early apoptosis was detected by Annexin⁺ve staining of immature and mature sperm. Exposure to STS only or in combination with 3-ABA did not show a significant difference in the percentage of early apoptotic sperm in both immature and mature sperm compared with the control group. When both immature and mature sperm were combined and examined for effects of STS + 3-ABA exposure, a 38% decrease in early apoptotic sperm percentage was seen compared with the STS-only group; however, this decrease was nonsignificant (Table 1).

Exposure to oxidative stress by H₂O₂ caused a significant decrease in the percentage of early apoptotic sperm in immature and mature sperm (0.91% ± 0.58% [P = .039] and 0.33% ± 0.36% [P = .008], respectively) compared with the control group. Exposure to H₂O₂ + 3-ABA resulted in a significant increase in the percentage of early apoptotic sperm in immature (1.28 ± 0.39%; P = 0.039) and mature (0.59 ± 0.52%; P = .008) sperm compared with the control group. When both immature and mature sperm were combined, a significant increase was seen in the percentage of early apoptotic sperm exposed to H₂O₂ + 3-ABA versus the H₂O₂-only group (P = .036; Table 1).

**Late apoptosis** Immature sperm showed a higher incidence of late apoptotic sperm in the control, STS-only, and STS + 3-ABA groups compared with the mature sperm fractions. A significantly higher percentage of late apoptotic sperm was seen in the immature sperm in the STS-only group (P = .039). Within the immature and mature sperm fractions, no difference was seen in the percentage of late apoptotic sperm in the STS-only group, whereas a significantly higher...
percentage was seen in the STS + 3-ABA treated group in immature ($P = .016$) and mature ($P = .023$) sperm fraction compared with the control group (Fig. 4).

Mature sperm fraction showed a higher incidence of late apoptosis after exposure to both H$_2$O$_2$ only and H$_2$O$_2$ + 3-ABA compared with immature sperm ($P = .016$ and $P = .014$, respectively). Within immature and mature fractions, the immature fraction showed a higher incidence of late apoptotic sperm in the H$_2$O$_2$-only and H$_2$O$_2$ + 3-ABA exposed groups compared with the control group ($P = .039$ and $P = .038$, respectively; Fig. 4). Similarly, a higher incidence of late apoptotic sperm was seen in the mature fraction for H$_2$O$_2$ -only and H$_2$O$_2$ + 3-ABA exposed groups compared with the control group ($P = .008$ and $P = .008$, respectively; Fig. 4).

Combined immature and mature sperm fractions and STS + 3-ABA treatment showed a significantly higher incidence of late apoptotic sperm ($P < .001$; Table 1) compared with STS-only groups. Exposure to H$_2$O$_2$ + 3-ABA showed a non-significant decrease in the percentage of late apoptotic sperm compared with the H$_2$O$_2$-only group (Table 1).

**Caspase Activation by Active Caspase-3 Assay**

No significant changes in active caspase-3 level were seen in either immature or mature sperm exposed to STS only, H$_2$O$_2$ only, or in the presence of 3-ABA.

**Correlation Between cPARP, TUNEL, Apoptosis, and Active Caspase-3**

Apoptotic markers (cPARP, TUNEL, early and late apoptosis, and active caspase-3) were examined for their association with chemical (STS only and STS + 3-ABA) or oxidative (H$_2$O$_2$ only and H$_2$O$_2$ + 3-ABA) stress treatment (Table 2). Significant correlations were seen with cPARP in all treated groups (Table 2).

In the untreated group, a positive correlation ($r = 0.56$; $P = .02$) was observed between the percentages of sperm positive for cPARP and those that were positive for active caspase-3 in the combined (mature and immature) sperm fraction. This correlation became stronger in the STS-only and STS + 3-ABA treated groups ($r = 0.72$ and $r = 0.74$; $P = .002$ and $P < .001$, respectively). In the immature fraction, the percentage of cPARP$^+$ve sperm showed a positive correlation with active caspase-3$^+$ve sperm in the STS + 3-ABA (r = 0.83; $P = .015$), H$_2$O$_2$ only (r = 0.74; $P = .046$), and H$_2$O$_2$ + 3-ABA (r = 0.73; $P = .046$) groups.

**DISCUSSION**

We have demonstrated detectable levels of cPARP in ejaculated human mature and immature spermatozoa by flow
cytometry. Our present study puts to rest the controversial de-
bate on the presence (53) or absence (25, 68) of PARP.

Poly(ADP-ribose) polymerase is involved in gamete dif-
ferentiation during spermatogenesis (51), spermiogenesis
(52), and sperm chromatin remodeling (49).

Our findings are in agreement to those of Maymon et al.
(51), who reported increased PARP-1 expression in all types
of spermatogonia in different maturation stages. Those inves-
tigators suggested that increased PARP-1 expression may act
as a reservoir for maintaining DNA integrity during germline
differentiation. Our previous study (54), in which we reported
the presence of PARP homologues in mature and immature
spermatozoa, supports this observation. Poly(ADP-ribose)
cleavage may be involved in the DNA damage/repair process,
because its incidence is seen to increase with progression of spermatogenesis (48). The higher, although

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Measured Parameters in Different Modulation Treatments Without and With PARP Inhibitor (3-ABA).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>%, Mean ± SD</td>
</tr>
<tr>
<td>TUNEL+ve</td>
<td>STS only</td>
</tr>
<tr>
<td>STS + 3-ABA</td>
<td>11.6 ± 7.9</td>
</tr>
<tr>
<td>H2O2 only</td>
<td>11.1 ± 6.9</td>
</tr>
<tr>
<td>H2O2 + 3-ABA</td>
<td>12.2 ± 9.4</td>
</tr>
<tr>
<td>cPARP</td>
<td>STS only</td>
</tr>
<tr>
<td>STS + 3-ABA</td>
<td>44.3 ± 28.7</td>
</tr>
<tr>
<td>H2O2 only</td>
<td>47.5 ± 28.2</td>
</tr>
<tr>
<td>H2O2 + 3-ABA</td>
<td>48.7 ± 29.1</td>
</tr>
<tr>
<td>Late apoptotic sperm</td>
<td>STS only</td>
</tr>
<tr>
<td>STS + 3-ABA</td>
<td>75.5 ± 16.9</td>
</tr>
<tr>
<td>H2O2 only</td>
<td>90.9 ± 22.9</td>
</tr>
<tr>
<td>H2O2 + 3-ABA</td>
<td>88.9 ± 24.7</td>
</tr>
<tr>
<td>Early apoptotic sperm</td>
<td>STS Only</td>
</tr>
<tr>
<td>STS + 3-ABA</td>
<td>4.9 ± 4.7</td>
</tr>
<tr>
<td>H2O2 Only</td>
<td>0.62 ± 0.55</td>
</tr>
<tr>
<td>H2O2 + 3-ABA</td>
<td>0.93 ± 0.81</td>
</tr>
</tbody>
</table>

Note: 3-ABA = 3-aminobenzamide; cPARP = cleaved poly(ADP-ribose) polymerase; H2O2 = hydrogen per-
oxide; PARP = poly(ADP-ribose) polymerase; PI = propidium iodide; TUNEL = terminal deoxynucleo-
tidyl transferase–mediated fluorescein-dUTP nick-
end labeling.

*P value with and without 3-ABA. P < .05 was considered to be significant.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Correlation Between Each Measured Parameter in Different Modulation Treatments Without and With PARP Inhibitor (3-ABA).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>r</td>
</tr>
<tr>
<td>Activated caspase-3+ spermatozoa</td>
<td>STS only</td>
</tr>
<tr>
<td>STS + 3-ABA</td>
<td>0.98</td>
</tr>
<tr>
<td>H2O2 only</td>
<td>0.95</td>
</tr>
<tr>
<td>H2O2 + 3-ABA</td>
<td>0.42</td>
</tr>
<tr>
<td>TUNEL+ spermatozoa</td>
<td>STS only</td>
</tr>
<tr>
<td>STS + 3-ABA</td>
<td>0.68</td>
</tr>
<tr>
<td>H2O2 only</td>
<td>0.64</td>
</tr>
<tr>
<td>H2O2 + 3-ABA</td>
<td>0.64</td>
</tr>
<tr>
<td>cPARP+ spermatozoa</td>
<td>STS only</td>
</tr>
<tr>
<td>STS + 3-ABA</td>
<td>0.88</td>
</tr>
<tr>
<td>H2O2 only</td>
<td>0.82</td>
</tr>
<tr>
<td>H2O2 + 3-ABA</td>
<td>0.83</td>
</tr>
<tr>
<td>Early apoptotic sperm (annexin V+ spermatozoa)</td>
<td>STS only</td>
</tr>
<tr>
<td>STS + 3-ABA</td>
<td>−0.13</td>
</tr>
<tr>
<td>H2O2 Only</td>
<td>−0.48</td>
</tr>
<tr>
<td>H2O2 + 3-ABA</td>
<td>−0.20</td>
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<tr>
<td>Late apoptotic sperm (annexin V/PI+ spermatozoa)</td>
<td>STS Only</td>
</tr>
<tr>
<td>STS + 3-ABA</td>
<td>0.37</td>
</tr>
<tr>
<td>H2O2 only</td>
<td>−0.28</td>
</tr>
<tr>
<td>H2O2 + 3-ABA</td>
<td>−0.13</td>
</tr>
</tbody>
</table>

Note: P < .05 was considered to be significant. PI = propidium iodide; other abbreviations as in Table 1.

nonsignificant, levels of cPARP reported in the present study in the mature sperm fraction is supported further by the increased band density of PARP homologues that we previously reported for the first time in mature sperm of proven fertile donors (54). The present study findings indicate a potential role of PARP during sperm maturation and the sperm chromatin remodulation processes. This observation is also supported by several other recent reports (49, 69, 70).

Poly(ADP-ribose) polymerase 1 plays a role in both necrotic and apoptotic cell death pathways, as illustrated in Figure 1. Hypersynthesis of PAR by PARP-1 in response to extensive DNA damage causes depletion of nicotinamide-adenine dinucleotide (NAD) and ATP, leading to energy failure and cell necrosis (71). Poly(ADP-ribose) polymerase 1 can promote a caspase-independent apoptotic pathway through apoptosis-inducing factor (72). Although the mechanism underlying the choice between apoptosis and necrosis in response to genotoxic stimuli is unclear, it may be affected by cell type, stimuli type and strength, and duration of exposure (73).

The present study findings support the above hypothesis of the cell death pathway(s). In the absence of PARP inhibition, exposure to STS only resulted in a large increase in the percentage of early apoptotic sperm. This increase in early apoptotic sperm with STS declined after PARP inhibition. Furthermore, we obtained a >2-fold increase in the percentage of late apoptotic sperm after PARP inhibition in STS-induced sperm injury. The increase in late apoptotic sperm with chemical exposure and PARP inhibition are more apparent in immature than in mature sperm fractions, suggesting mature spermatozoa are more protected from chemical-induced damage. This may explain the possible therapeutic application of PARP in selectively facilitating tumor cell death alone or in combination with chemotherapy (74–76) or viral infection (77, 78).

On exposure to oxidative stress, PARP inhibition caused a significant increase only in the percentage of early apoptotic sperm (Table 1). Immature spermatozoa show more resistance to this damage compared with mature sperm, suggesting that mature sperm are more susceptible to oxidative stress. The oxidative stress–induced increase in early apoptotic sperm after PARP inhibition also suggests that overactivation of PARP can increase late apoptosis and necrosis. This is also evident by reports of DNA breaks after oxidative stress causing overactivation of PARP and promoting cell dysfunction/necrosis in diabetes mellitus, stroke, and cardiovascular diseases (74, 79).

The present study findings show cPARP to be correlated with activated caspase-3. Poly(ADP-ribose) polymerase
cleavage is catalyzed by activated caspase-3 which initiates apoptosis and prevents PARP-mediated DNA repair processes. Inactivation of PARP has been proposed to prevent depletion of NAD (a PARP substrate) and preserve ATP (80). Earlier reports have documented apoptosis via oxidative stress (81) and chemical induction (82) through caspase-dependent and -independent pathways. One of the mechanisms through which oxidative stress or tumor necrosis factor alpha mediates cell death is activation of transient receptor potential cation channel, subfamily M, member 2 (TRPM2). This results in increased intracellular Ca++ followed by caspase activation and PARP cleavage (83).

Spermatocyte function is reported to be affected by PARP-1 or PARP-2 automodification (84). We hypothesized that sperm with reduced levels of cPARP might be deficient in postgestational protection/repair during exposure to a sperm-damaging agent. This PARP-dependent mechanism may be inefficient in infertile men; therefore, the sperm are more susceptible to damage. In our previous study, we identified the presence of different PARP homologues in ejaculated sperm (54). Poly(ADP-ribose) polymerase homologues have a variety of molecular and cellular functions. Poly(ADP-ribose) polymerase 1 and PARP-2 are involved in DNA repair mechanisms. (39, 40, 52, 73, 85).

Collectively, mature and immature spermatozoa showed a significant decline in the percentage of late apoptotic sperm after PARP inhibition in chemical and oxidative stress–induced sperm damage. The response of oxidative stress–induced damage to PARP inhibition was low compared with that of chemical damage. This suggests that oxidative stress–induced sperm damage may have different pathway(s), such as peroxidation of lipids and proteins, in addition to DNA damage. This was evident from the increase in early apoptotic sperm seen in oxidative stress–induced damage even after PARP inhibition.

Our study limitation was the small sample size; however, this was a pilot study. We recommend additional studies with a large sample size. In addition, it will be important to examine DNase-induced injury to ensure direct DNA damage of sperm after exposure to other injurious agents. Optimizing the duration and concentration of exposure to these toxic agents and PARP inhibitors is also important. Furthermore, it would be interesting to examine the type of PARP homologue cleaved by these treatments, thus opening new therapeutic applications for infertile patients. As we reported in our earlier study PARP-2 may play a role in sperm damage response and additional studies are needed to explain role of PARP-9 (54).

In conclusion, cPARP is a new apoptotic marker that can be detected in ejaculated spermatozoa by flow cytometry and may be related to the activation of caspase-3. Both immature and mature sperm show cPARP expression after chemical or oxidative stress–induced damage. Poly(ADP-ribose) polymerase inhibition can modulate the incidence of early or late apoptosis in ejaculated human spermatozoa.

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