Title: IN VITRO ANALYSIS OF THE EFFECT OF BENZO-α-PYRENE ON SPERM HYPERACTIVATION

Objective: Tobacco combustion yields about 4000 compounds and one of the most harmful
components of it is benzo-α-pyrene. The aim of this study was to evaluate the in-vitro effect of benzo-α-pyrene on sperm hyperactivation in normozoospermic semen samples of non-smokers analyzed by CASA (computer-assisted-semen-analysis).

**Design:** Prospective study.

**Materials and Methods:** Spermatozoa from 13 normal fertile, normozoospermic, nonsmoking donors, washed free of seminal plasma, were treated with medium (HTF) alone (control); and, with different concentrations of benzo-α-pyrene. The benzopyrene concentrations were: 100 microgram/mL (4B100), 50 microgram/mL (3B50), 25 microgram/mL (2B25) and 12.5 microgram/mL (1B12.5). CASA (Model: Sperm Class Analyzer, version: 3.2.0, Barcelona, Spain) was done at 1 hr after incubation. The acrosome status of these treated samples was analyzed by gelatin halo test.

<table>
<thead>
<tr>
<th>Group / concentration (microgram/mL)</th>
<th>mean hyperactivation % (mean ±SD)</th>
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</thead>
<tbody>
<tr>
<td>control</td>
<td>2.12 ± 0.66</td>
</tr>
<tr>
<td>1B (12.5)</td>
<td>5.8 ± 5.53</td>
</tr>
<tr>
<td>2B (25)</td>
<td>14.67 ± 9.79</td>
</tr>
<tr>
<td>3B (50)</td>
<td>19.47 ± 12.87</td>
</tr>
<tr>
<td>4B (100)</td>
<td>28.40 ± 22.42</td>
</tr>
</tbody>
</table>

P < 0.05 on control vs 3B50 and p < 0.01 on control vs 4B100

**Results:** benzo-α-pyrene at concentrations of > or = 50 microgram/mL significantly increased sperm hyperactivation. The % hyper-activation (mean ±SD) in each group was as follows; control- 2.12±0.66, 1B12.5- 5.8±5.53, 2B25- 14.67±9.79, 3B50- 19.47±12.87, 4B100- 28.40 ±22.42. Acrosome status of the benzo-a-pyrene treated sperms was monitored by evaluating the level of halo formation in the gelatin based acrosome halo test. More than 82% sperms were acrosome reacted at concentrations > 50 microgram/ml.

**Conclusions:** Sperm hyperactivated motility is characterized by high flagellar bend amplitude and asymmetrical beating, which are detected by computer-assisted sperm motility analysis as increased curvilinear velocity and lateral head movement. It is required for sperm penetration of the oocyte zona pellucida during fertilization and is induced by an increase in flagellar Ca²⁺. This study proves that the increasing hypermotility of the sperms occur with increasing concentrations of benzo-α-pyrene, an active component of tobacco, which is a result/concomitant of premature, false, acrosomal reaction. benzo-α-pyrene increased sperm hyperactivation significantly which result in premature acrosomal reaction.

**Support:** None.

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