Reproductive toxicity of cyto-static drugs and pharmacological ways to reduce it

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<table>
<thead>
<tr>
<th>Disease</th>
<th>Scheme</th>
<th>Status of reproductive function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Cancer</td>
<td>CMF, FAC</td>
<td>amenorrhea in <strong>88%</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>amenorrhea in <strong>55%</strong></td>
</tr>
<tr>
<td>Hemoblastosis</td>
<td>COPP, EEACOPP, CHOP, ABVD</td>
<td>amenorrhea in the majority of women</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Violations in ovarian cycle are minimal</td>
</tr>
<tr>
<td>Ovarian cancer (after conserving surgery)</td>
<td>POMB/ACE</td>
<td>Does not result in sterilization</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Group and name of the drug, chemical structure</th>
<th>Main mechanism of anti-tumor action</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PLATINUM COMPLEXES</strong></td>
<td></td>
</tr>
<tr>
<td>Cisplatin, Lachema AC, Austria</td>
<td>Form a cross-link between DNA molecules</td>
</tr>
<tr>
<td>Carboplatin, EBEWE Pharma, Austria</td>
<td></td>
</tr>
<tr>
<td><strong>ANTHRACYCLINE ANTIBIOTICS</strong></td>
<td></td>
</tr>
<tr>
<td>Doxorubicin, EBEWE Pharma, Austria</td>
<td>Intercalation between the base pairs of DNA</td>
</tr>
<tr>
<td>Epirubicin, Karlo Arba, Italy</td>
<td></td>
</tr>
<tr>
<td><strong>INHIBITORS TOPOIZOMERAZNOY ACTIVITY</strong></td>
<td></td>
</tr>
<tr>
<td>Etoposide, Teva Pharmaceutical Industries, Israel</td>
<td>Inhibition of topoisomerase II</td>
</tr>
<tr>
<td><strong>TAXOIDS</strong></td>
<td></td>
</tr>
<tr>
<td>Paclitaxel, Dr Reddis, India</td>
<td>Stimulation of assembling of anomalous microtubules</td>
</tr>
</tbody>
</table>
The object of study is Wistar rats

The drugs were administered intravenously in a single MTD, because in clinics high-dose therapy is used

Methods of study

- **morphological**
  (using quantitative indicators characterizing extent of the damage)
- **functional**
  (fertility index, the index pregnancy, fetal death)

Research terms

The assessment of effects was performed 3 and 6 months after administration of cyto-static drugs
Early antiproliferative effects of cytotoxic drugs on gonads

On testicular tissue:
"DNA-comets" of mouse testis

A – cells with DNA-damages
B – Apoptopic "DNA-comets"

On ovarian tissue:
Death of follicular epithelium cells

Tubules with the 12th stage of meiosis, %
Number of normal spermatogonia, % of control

Primordial follicles

Control  Epirubicin  Etoposide  Doxorubicin  Cisplatin  Paclitaxel
Content of structural-functional elements of rats ovaries, 6 months after a single injection of anticancer drugs in the MTD (% of control)

Ep – Epirubicin; Cs – Cisplatin; Cr – Carboplatin; Et – Etoposide; P – Paclitaxel
Intensity of long-term-late effects of cyto-static drugs on structural and functional elements of the rat ovary is decreased in the following order:

1. Epirubicin
2. Etoposide
3. Paclitaxel
4. Cisplatin
5. Carboplatin
Efficiency of mating in female-rats in the long-term period after administration of cytotoxic drugs of different groups

Ep – Epirubicin; Cs – Cisplatin; Cr – Carboplatin; Et – Etoposide; P – Paclitaxel
Embryonic mortality in female rats while the crossbreeding long-term period after administration of cito-static drugs of different groups (% of control)

Ep – Epirubicin; Cs – Cisplatin; Cr – Carboplatin; Et – Etoposide; P – Paclitaxel
Toxic effect of drugs on embryonic mortality is decreased in the following order:

1. Taxanes
2. Inhibitors of topo-isomerase activity
3. Anthracycline antibiotics
4. Platinoids
Morphological status of the testes of rats at 3 months after administration of Paclitaxel and Epirubicin

Intact rat testis, age 5.5 months, x160. Staining with hematoxylin and eosin.

Testis rats 3 months after administration of Paclitaxel and / or Epirubicin, x160. Thinning seminiferous epithelium. Staining with hematoxylin and eosin.
Sperm count, and efficiency of mating male rats at 3 months after administration of cyto-static drugs of different groups

Ep – Epirubicin; Cs – Cisplatin; Cr – Carboplatin; Et – Etoposide; P – Paclitaxel
## State of reproductive system of male rats long-term after administration of cyto-static drugs of different groups

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sexual instinct</th>
<th>Fertility</th>
<th>Level of (DLM) (characterizes the probability to save pregnancy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platidiam</td>
<td>Not disturbed</td>
<td>Not disturbed</td>
<td>Not increased</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>Not disturbed</td>
<td>Not disturbed</td>
<td>Not increased</td>
</tr>
<tr>
<td>Pharmorubicin</td>
<td>Not disturbed</td>
<td>Infertility, 100%</td>
<td>Not increased</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>Not disturbed</td>
<td>Not disturbed</td>
<td>Not increased</td>
</tr>
<tr>
<td>Etoposide</td>
<td>Not disturbed</td>
<td>Not disturbed</td>
<td>Increased</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>Not disturbed</td>
<td>Infertility, 100%</td>
<td>Increased</td>
</tr>
</tbody>
</table>

**Toxicity decreases in the following order:**

- Pharmorubicin
- Paclitaxel
- Etoposide

In platinum drugs toxicity was not found.
Possible ways to reduce the long-term consequences of the effect of cyto-static drugs on reproductive system by assisting reproductive technologies

- **Cryopreservation of sperm**
- **Testis tissue biopsy**
- **Cryopreservation of oocytes**
- **Cryopreservation of ovarian tissue**
- **Cryopreservation of embryos**
- **Differentiation of bone marrow stem cells into male germ cells**

Negative aspects of assisted reproductive technologies:
1. High cost
2. Inability to perform due to the need to start chemotherapy
3. High sensitivity of oocytes to freezing

Comments:
- IVF - in vitro fertilization
- ISI - Intracytoplasmic Sperm Injection
- ЧССК - human spermatogonial stem cell
The effectiveness of drug therapy as the way to reduce the effects of cyto-static gonadotoxicity

- **Gonadal-hormone products**
  - Stimulator of spermatogenesis (testosterone)
  - Hypothalamic regulators of pituitary function

- **Immunomodulators**
  
- **Drugs limiting apoptosis in oocytes** (sfignozin monophosphate)

- **Means of regenerative medicine**

- **Antioxidants**

Negative aspects:
1. High cost
2. Inability to perform due to the need to start chemotherapy

References:
- [Tilly J.L. et al., 2004]
- [Delis J. et al., 1987]
- [Bocker L. et al., 1990; Borovskaya T.G. et al., 2007]
- [Kolomietz O.L. et al., 2001; Borovskaya T.G. et al., 2003]
- [Carmely A., 2009]
- [Tilly J.L. et al., 2004; Borovskaya T.G., Dygai A.M., Zhdanov V.V., 2008]
Number of structural and functional elements of rats ovaries, 6 months after combined administration of Etoposide and Buserelin

% of control (etoposide)

- Primordial follicles
- Double and multiple follicles
- Atretic follicle
- Yellow body
- Graafian follicles
Recent years, the new information about the properties of pluripotent progenitor cells of the body was obtained. The possibility of mobilizing the internal mechanisms of "deep reserve" – bone marrow stem cells and their following homing into the damaged tissue and activation of regional stem cells by various cytokines is shown.

A.M. Dygai, V.V. Zhdanov et al. 2006, 2010, 2011
Reparative regeneration of testicular tissue after administration of Paclitaxel

Restoring of spermatogonia goes under upgrading of spermatogenic layer

- Stem spermatogonia
- Cell microenvironment - Sertoli cells
- Rete testis
- Immature seed tubule
- Mature seed tubule
- Proxenouski Leydig
Status of spermatogenesis in rats late after combined administration of paclitaxel with G-CSF and pegylated G-CSF

Degree of maturity of spermatogenic layer (a.u.)

Amount of spermatogonia (% of background)

Total amount of germ cells (% of background)

- differences are significant compared to the background  
- differences are significant compared to the control
Effect of antioxidant from the group of sterically hindered phenols to the level of DNA comets in the testes of mice treated with methyl-meta-sulphonate or paclitaxel
Thank you for attention!