CONTRIBUTIONS ON THE ATTENUATE OF THE CRYOGENICS RESPONSE OF CONSTITUENTS PROTEINS HOMEOSTASIS OF THE SEMEN MATERIAL

CONTRIBUȚII ÎN ATENUAREA ECOURILOR CRIOGENICE ALE HOMEOSTAZIEI PROTEINELOR CONSTITUENTE ÎN MATERIALUL SEMINAL

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The evidence of cryogenics response of the semen proteins, the influence of BioR administration on homeostasis of constituent gametes proteomics and on the cryobiological indexes of bull semen material was studied. The investigation has been performed on bulls from the Black Spotted breed of Moldavian type, maintained during the investigation in adequate conditions from the point of view of microclimate and fodder. The biopreparation administration have been done daily during 10 days in volume of 0,2 ml/100 kg living mass/day. Structural proteins of gametes posed the resistance given the influence of ultra low temperature (-196°C), content of totals proteins in the bull semen material denote no difference between the value of this parameter in the raw and cry preserved-thawed bull gametes. Both, in the raw and thawed semen cells the most rate occupy the hydrophilic proteins. After semen conservation-thawing process, it was observed a tendency of the diminution of hydrophilic proteins (-3.35%) and an increase of the basophilic proteins (+2.78 %). In the raw gametes prevail γ-globulins rate; conservation and thawing process of the semen material was associated by an increase of the albumins rate (+34.63%) in semen cells; the rate of other three proteomic fractions: α-, β- and γ-globulins was decreased given theirs value registered in raw gametes. After the intramuscular administration of BioR preparation during 10 days on the sire bulls have been certified any modification of the studied proteomic fractions rate in thawed bull semen cells; albumins rate was decreased with 30,14%, the γ-globulins rate was increased with 19,28% in the experimental group; the β- and α-globulins with 8,5% and 2,36%, respectively, given control group. The BioR has an evident influence on the cryobiological specifics features of spermatozoids, such as the seminal cells mobility, the longevity and the survival absolutly index what are intensely influenced.

Key words: the semen cells, the proteomic fractions, cryoconservation, semen thawing, the cryobiological indexes, the cryogenics response, bull semen material.
Introduction

The applied part of cryobiology – cryoconservation become a current biotechnological method in the animal reproduction, large used in dairy cattle branch, but no and in the other livestock species. One of the determinative factors, which limit integration of semen material cryoconservation in the sheep, pig, poultry, Artificial Insemination technology are the inferiority of the cryobiological indexes and a reduced fertilization rate of the thawing semen given the raw or refrigerated.

Generalization and analyses of the literature data [3; 6] and our long-term results [3; 5] on the semen cryobiology justify the thoroughgoing the study concerning the cryogenics echo of the farm animals male gametes at different levels of their structural organization and the elaboration the efficient proceedings of increase of the semen cryocompetitiveness, both on the spermatogenesis stage and during conservation-thawing procedures.

The purpose of our investigation – evidence of cryogenics response of the semen proteins and the influence of BioR administration on the proteomics homeostasis and on the cryobiological indexes of bull semen material.

Materials and Methods

The investigation has been performed on bulls from the Black Spotted breed of Moldavian type, maintained during the investigation in adequate conditions from the point of view of microclimate and fodder.

The biopreparation administrations have been done daily during 10 days in volume of 0.2 ml/100 kg living mass/day. Having as purpose the research of the cryoconservation influence on the bull semen proteomics and the effect of BioR administration on the semen proteomics homeostasis and on the cryobiological semen indexes, in the control and experimental groups have been studied: the mobility, the longevity and the survival absolutely indexes in the raw and thawed bull semen; the metabolic indexes, such as content of proteins in the raw and cryopreserved semen. Total proteins and proteomics fractions was determined by [1; 4], utilizing the preparation produced by “Lyonorm” and “La Chemia” (Brno), “Biocont” (Russia). Indexes of semen quality were determined by general accepted method in the laboratory for semen microscopic examination [2].

Results and Discussions

Results of our experiments concerning the content of total proteins in the raw and thawed bull semen material (diag. 1) denote no difference between value of this indices in the raw and cryopreserved-thawed bull gametes.
By the same token, the structural gametes proteins posed the resistance given the influence of ultra low temperature (-196°C).
On further in the raw gametes the rate of some proteins fractions in accordance with their izoelectric point and the consequents of the thermal stress caused by the cryoconservation process have been studded (tab. 1).
The obtained experimental data indicate that both in the raw and thawed semen cells the most rate occupy the hydrophilic proteins. The lowest rate was own the basophilic fraction. The acidophilic proteins occupy a middle position in this rate hierarchy. Similar to totals proteins, after semen conservation-thawing process neither of theirs (tab.1) support the significantly modifications. It was observed a tendency of the diminution of the hydrophilic proteins (- 3,35%) and an increase of the basophilic proteins (+ 2,78 %).

Table 1

<table>
<thead>
<tr>
<th>Specification</th>
<th>Hydrophilic proteins</th>
<th>Basophilic proteins</th>
<th>Acidophilic proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw semen material</td>
<td>5,39 ±0,87</td>
<td>2,21±0,22</td>
<td>3,79±0,38</td>
</tr>
<tr>
<td>Thawed semen material</td>
<td>4,97±0,65</td>
<td>2,51±0,40</td>
<td>3,83±0,23</td>
</tr>
<tr>
<td>± given raw semen, %</td>
<td>-3,35</td>
<td>+2,78</td>
<td>+0,11</td>
</tr>
</tbody>
</table>

Results of the albumins, α-, β- and γ-globulins fractions content in the raw and cryopreservated-thawed bull semen cells is presented by diag. 2 and diag. 3.
The analysis of the studded proteins fractions has evaluated that in the raw gametes prevail γ-globulins. Conservation and thawing process of the semen material was associated by an increase of albumins rate. The rate of other tree proteomic fractions: α-, β- and γ-globulins was decreased given theirs value registered in the raw gametes.

The influence of the intramuscular administration of BioR preparation during 10 days in the sire bulls on the albumins, α-, β- and γ-globulins fractions content is presented in the diag. 4. The obtained experimental results in these investigation certify any modification of the studded proteomic fractions rate in thawed bull semen cells, given control (semen cells from non treated by BioR bulls). The γ-globulins rate was increased with 19,28% in the experimental group. Also, it was observed an increase of β- and α-globulins globulins with 8,5% and 2,36%, respectively, given control group.

In the same condition the value of albumins rate, in the thawed semen cells of experimental bulls was decreased with 32,86% given non treated with BioR preparation (diag. 4).

Concomitantly, we have studded the mobility, the longevity and the survival absolutly index of thawing gametes (tab. 2).

Analize of presented date (tab.2) notice that the BioR administration was contributted on the significantly cryobiological characteristics increase of thawing semen material. The highest result was inscribed counterpart the survival absolutly index and the mobility of the thawed gametes of sire bulls. In case of the longevity it was established a temperate increase. In accordance with the established results of modifications of the proteomic fractions rate in semen cells
The proteomic fractions rate,%

<table>
<thead>
<tr>
<th>Albumins</th>
<th>α-Globulins</th>
<th>β-Globulins</th>
<th>γ-Globulins</th>
</tr>
</thead>
<tbody>
<tr>
<td>46,98</td>
<td>22,22</td>
<td>18,04</td>
<td>21,26</td>
</tr>
</tbody>
</table>

Diag. 4. The influence of BioR on the proteomic fractions rate in bull semen cells

Table 2. The influence of BioR administration on the cryobiological indexes of semen material

<table>
<thead>
<tr>
<th>Nr.</th>
<th>The experimental groups</th>
<th>Mobility, points</th>
<th>Longevity, hours</th>
<th>Survival Absolutely Index, c.u.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>I (control)</td>
<td>3.43 ± 0.17</td>
<td>8.33 ± 0.67</td>
<td>11.08 ± 1.29</td>
</tr>
<tr>
<td>2.</td>
<td>II (0.2 ml)</td>
<td>4.21 ± 0.27*</td>
<td>9.57 ± 0.20</td>
<td>18.64 ± 2.73*</td>
</tr>
<tr>
<td>3.</td>
<td>± given control</td>
<td>+ 22.74</td>
<td>+ 14.88</td>
<td>+ 68.23</td>
</tr>
</tbody>
</table>

P < 0.05;

In raw and thawed semen material, the influence of BioR administration on proteomic fractions rate in semen cells and on the cryobiological parameters of thawed bull semen is possible to mention that BioR have been attenuate the cryogenics response of some semen cells proteomic fractions and consecutively increase the cryobiological characteristics of the thawed bull semen. Since thus parameters are a high influence on the spermatozoids skill fecundity it can to conclude that the BioR will increase the rate of the fecundity of females after first artificial insemination.
Conclusions

1. Structural gametes proteins posed the resistance given influence of ultra low temperature (-196°C), content of total proteins in the raw and thawed bull semen material denote no difference between value of this parameter in the raw and cryopreserved-thawed bull gametes.

2. Both in the raw and thawed semen cells the most rate occupy the hydrophilic proteins, after semen conservation-thawing process, it was observed a tendency of the diminution of hydrophilic proteins (-3.35%) and an increase of the basophilic proteins (+2.78%).

3. In the raw gametes prevail γ-globulins content; conservation and thawing process of the semen material was associated by an increase of the albumins rate in semen cells; the rate of other three proteomic fractions - α-, β- and γ-globulins was decreased given to theirs value registered in raw gametes.

4. After the intramuscular administration of BioR preparation during 10 days on the sire bulls in semen have been certify significantly modification on the content of albumins (-30.14%) in thawed bull semen cells; the γ-globulins rate was increased with 19.28% in the experimental group; the β- and α-globulins with 8.5% and 2.36%, respectively, given control group.

5. The BioR has an evident influence on the cryobiological specifics features of spermatozoids, such as the seminal cells mobility, the longevity and the survival absolutely indice are intensely influenced.

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