Tangent Contribution in the Increase of Efficiency of Carp’s Reproduction Technology Through in vitro Fecundation Method

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Abstract
The influence of the BioR preparation on some indices of sperm quality, spawn fertilization ability and early embryo development of carp under the conditions of in vitro fertilization was studied. During the experiment there have been chosen ordinary carp (males and females at the age of 5-6 years) used in carp artificial reproduction sector. The injection of BioR to reproductive carp in the period of preparation for artificial reproduction contributed to: - a significant increase of sperms longevity after collection; - moderate improvement of seminal cells mobility after collection in the experimental group by 5.53% versus the control group; - the increase in seminal cells survival under conservation conditions for a short period of time at temperatures 0- +4°C. BioR favours in vitro fertilization results by improving functional indices of the seminal material, as well as spawn biological value

Keywords: biological semen indices, embryonic development, microalgal preparation, males and females of carp artificial reproduction nucleus, spawn fertilization

1. Introduction
Carp artificial fertilization at industrial scale is used in production farms, it is important in order to obtain fertilized spawn from known producers, when obtaining hybrids, when refreshing blood, when applying consanguinity, when testing acclimatization capacity etc. Anyway, the enterprises that are involved in fish reproduction by means of in vitro fertilization may have some difficulties caused by maturity and the collection of sexual products (spawn and sperms) asynchronization. In other cases the seminal material collected from male reproducers is of low quality that could put fertilization at risk. Sperm mobility and viability represent an important factor in spawn fertilization. That’s why the exploration of the possibilities of sperm survival duration has practical importance in the case of spawn artificial fertilization.

The objective is to study the influence of the biopreparation BioR on some indices of sperm quality and spawn fertilization ability of carp under the conditions of in vitro fertilization.

2. Materials and methods
The researches were made during the reproduction period in the fish enterprise of Telenesti. During the experiment there have been chosen ordinary carp (males and females at the age of 5-6 years) used in carp artificial reproduction sector.

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Gametogenesis stimulation has been carried out by means of intramuscular injections of pituitary solution in the dorsal side between the beginning of the dorsal fin and lateral line at a depth of about 2 cm, 4-6 mg of dry pituitary gland for females and 2 mg per 1 kg of weight.

For this purpose we have evaluated the influence of the biosynthesis remedy BioR injection on sperms mobility and survival duration. The preparation has been carried out by means of intramuscular injection in volume of 1ml/day, in one dose only, 5 days yearly of the pituitary stimulation.

**Results and discussion**

Sperm mobility and viability represent an important factor in spawn fertilization. That’s why the exploration of the possibilities of sperm survival duration has practical importance in the case of spawn artificial fertilization. In table 1 the results of the microalgal preparation testing are presented.

<table>
<thead>
<tr>
<th>Specification</th>
<th>Analysed samples (n)</th>
<th>Mobility, (Points)</th>
<th>Semen cells longevity, (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>5</td>
<td>6.87 ± 0.21</td>
<td>3.05 ± 0.20</td>
</tr>
<tr>
<td>Experimenta group (BioR)</td>
<td>5</td>
<td>7.25 ± 0.13</td>
<td>4.15 ± 0.25</td>
</tr>
</tbody>
</table>

The analysis of the obtained data (table 1) shows that, when administrating BioR to reproductive carp, we can observe the tendency of mobility distinction between the control group and the experimental group. Thus, the mobility of the seminal cells immediately after collection in the control group constitutes 6.78 points. In the experimental group sperms mobility after collection has increased and reached 7.52 points. Consequently, BioR injection has improved seminal cells mobility in reproductive carp by 5.53% in comparison with the control group.

BioR injection to reproductive carp during the period of preparation for artificial production has influenced seminal cells longevity after collection. Thus, in the control group the duration of vital activity manifest by the active movements of the sperms constitutes 3.05 minutes. This index grew to 4.15 minutes in the control group. Thus, the value of sperms’ vital activity after collection in the control group prevails over the experimental group by 36.06 %.

Sperm conservation at low temperatures was first mentioned in 1886 by Ovseanikov [cited by 1], [2]. Since then it has been used in fish farming. We can keep carp sperm alive for longer time if the sperm is conserved in a clean and dry vessel at low temperature (0- +4°C).

BioR injection to reproductive carp during the period of preparation for artificial production has influenced seminal cells longevity after collection. Thus, in the control group the duration of vital activity In this case it stays immobile, but once water or dilution medium is added, it gets active and keeps its fertilization capacity up to 8 days [3].

The results of BioRS injection influence on mobility dynamics, survival duration of seminal cells in the seminal material of the control and experimental groups are illustrated in Figure 1 and in Table 2.

**Figure 1. Influence of BioR injection on carp males on the seminal cells survival**

The data illustrated in Figure 1 show that the survival of carp seminal material under the conditions in vitro at temperature 0- +4°C differs in the experimental and the control groups. The best results have been established in the experimental group. Thus, sperm survival duration in the experimental group constituted 168.85 hours after collection. These results prevail over...
the control group by 16.73%. The data from the control group are lower than the data from specialty literature by 23.15 hours.

Consequently, there still exist chances for improvement in this domain.

Table 2. Influence of biosynthesis remedium BioRS on the mobility dynamics, survival duration of seminal cells preserved in vitro favours refrigeration (T= 0 - +4°C), n = 3

<table>
<thead>
<tr>
<th>Specification</th>
<th>collection</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
<th>144</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.87 ± 0.21</td>
<td>6.01±0.14</td>
<td>5.41±0.08</td>
<td>4.88±0.08</td>
<td>3.51±0.23</td>
<td>2.29±0.52</td>
<td>1.50±0.39</td>
<td>-</td>
</tr>
<tr>
<td>BioR</td>
<td>7.25 ± 0.13</td>
<td>6.81±0.41*</td>
<td>6.55±0.17*</td>
<td>5.13±0.17</td>
<td>4.25±0.18</td>
<td>3.15±0.18</td>
<td>2.05±0.28</td>
<td>0.55±0.15</td>
</tr>
<tr>
<td>± comparatively with control group, %</td>
<td>+ 5.53</td>
<td>+ 13.31</td>
<td>+ 21.07</td>
<td>+ 5.12</td>
<td>+ 21.08</td>
<td>+ 37.55</td>
<td>+ 36.67</td>
<td>-</td>
</tr>
</tbody>
</table>

Thus, after the collection we can observe an insignificant difference of the mobility of seminal cells in the experimental group in comparison with the control group. This difference constitutes only 5.53 %.

After 24 hours of seminal material conservation at temperatures 0 - +4° C, seminal cells mobility in the experimental group is higher by 13.31% than in the control group (P<0.5).

After 48 hours of seminal material collection and its conservation at temperatures 0 -+4° C the difference between the mobility of the seminal cells collected from reproductive carp of the experimental group and of the control group increased and constituted 21.01% (P<0.05).

After 72 hours of seminal material conservation through refrigeration the difference between the value of sperm mobility in the experimental group and the control group decreased and constitutes 5.12 % in favour of the experimental group.

After 96 hours of seminal material conservation the mobility of seminal cells in the experimental group prevails over the results of the control group by 21.08%.

This tendency is maintained after 120 and 144 hours of seminal material conservation, the difference for the experimental group is 37.55% and 36.67 % respectively.

Taking into consideration the data from specialty literature concerning competing fertilization factors in order to confirm the leading role of seminal cells quality in fertilization process [4, 5] we have evaluated this index using the sperm collected from the males of the experimental and control groups. The percentage of spawn fertility is illustrated in Figure 2.

Figure 2. Spawn fertilization in when the males of carp artificial reproduction nucleus was treated with BioR

The analysis of the obtained experimental results points out that BioR injection to reproductive carp has had tangent contribution by improving functional indices of the seminal material and to spawn fertilization increase. Thus, the rate of the fertilized spawn after 9 hours of mixing sexual products constituted 79.35 % in the control group.

We can observe moderate increase of the spawn fertilization results in the experimental group. Spawn fertilization rate in the experimental group prevails over the control group by 5.40 %.

Further we are going to expose the results of the evaluation of early embryonic development to the morula stage of carp embryos produced by in vitro fertilization. The evaluation of fertilization results first has taken place 9 hours after fertilization by
distinguishing 500 arbitrary roes in the field of vision and noting fertilized and non-fertilized roes. The data are illustrated in Figure 3.

Figure 3. The early embryonic development in the control and experimental group.

The data illustrated in Figure 3 show that embryos’ viability and their development capacity are different in the control group and the experimental group. Thus, in the control group early embryos and zygote losses at the zygote stage development-blastocyst constituted 27.59%. In group II, where we used for fertilization the seminal material collected from the reproducers injected with BioRs, the rate of viable embryos prevails over the control group by 8.12% and embryos ‘losses constitute 19.47% versus 27.59% in the control group.

4. Conclusions

1. The injection of BioR to reproductive carp in the period of preparation for artificial reproduction contributed to: -a significant increase of sperms longevity after collection; - moderate improvement of seminal cells mobility after collection in the experimental group by 5.53% versus the control group; - the increase in seminal cells survival under conservation by refrigeration at temperatures 0- +4°C.
2. BioR favours in vitro fertilization results by improving functional indices of the seminal material, as well as spawn biological value;
3. The best results of viability development capacity of early embryos have been registered in the experimental group.

References