Adding Motility Stimulants to Improve Freezing of Buffalo Sperm Recovered from Epididymal Cauda

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ABSTRACT

The cryopreservation of epididymal sperm is important to preserve genetic material from valuable buffalo bulls. This study evaluated the viability of post-thawed sperm samples recovered from the epididymal cauda adding motility inductors. For that, were used epididymides from eight Murrah buffaloes with 18 months of age. Semen samples were submitted to three different conditions: (CT - control) without adding medium, (SPERM) adding Sperm Talp medium, and (FERT) adding Fert Talp medium. Immediately after slaughter, both testicles from each animal were collected and transported at 4°C at maximum six hours interval. In laboratory, the removed epididymides was flushed to obtain sperm and diluted in the freezing extender. Each buffalo sperm were divided and fractions were submitted to all conditions (CT, SPERM and FERT). Semen doses were frozen at -196°C. CT, SPERM and FERT post-thawing results were 13.63±8.91, 38.77±8.91 and 42.83±8.91 for total motility, 7.30±8.74, 24.87±8.74 and 29.70±8.74 for progressive motility, 6.04±0.92, 6.74±0.92 and 6.93±0.92 for percentage of rapid cells (P < 0.05). In conclusion, diluted semen supplementation with Sperm or Fert talp increases the motility of cauda epididymal sperm of buffalo bulls.

Keywords: semen, buffalo, epididymal, freezing, motility, medium.

INTRODUCTION

The cryopreservation of epididymal sperm is important to preserve genetic material from valuable buffalo bulls. In other species, studies revealed an improvement of epididymal sperm quality after incubation with Sperm talp and Fert talp before freezing (Monteiro et al., 2012; 2011; Melo et al., 2010), because these mediums can stimulate semen motility. This study aimed to evaluate the viability of post-thawed sperm samples recovered from the epididymal cauda adding these motility factors.

MATERIALS AND METHODS

Were used epididymides from eight Murrah buffaloes with 18 months of age. Immediately after slaughter, both testicles from each animal were collected and transported at 4°C at maximum six hours interval.

In laboratory, the removed epididymis was flushed with to obtain sperm and diluted in Botu-Semen. Each buffalo sperm were divided and fractions were incubated under three different conditions: without adding medium (CT – control; Botu-Semen), adding Sperm Talp medium (SPERM; 80% Botu-Semen and 20% Sperm-talp), and adding Fert Talp medium (FERT; 80% Botu-Semen and 20% Fert-talp). After 15 minutes, samples were centrifuged and the pellets were resuspended in freezing extender Tris. For cryopreservation, the straws were maintained at 5°C/4h, and then 20 minutes at 3 cm above liquid nitrogen before immersion.

Post-thawed samples were evaluated by Computer-Assisted Semen Analysis (Hamilton Thorne HTM-IVOS 12, Beverly, MA) to verify % total sperm motility (MT), % progressive sperm

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motility (MP), velocity of trajectory (VAP; µm/s), linear progressive velocity (VSL; µm/s), curvilinear velocity (VCL; µm/s) and % rapid sperm (RAP). Fluorescent probes (CFDA and IP) were used to evaluate % plasma membrane integrity (IM).

For statistical analysis, data were evaluated by ANOVA (SAS, 2012). Differences between means were tested with Tukey test at 5% probability.

RESULTS AND DISCUSSIONS

SPERM and FERT groups post-thawing results were significant better than CT group on MT, MP, VAP, VSL, VCL and IM parameters. Motilities results were 13.63±8.91%, 38.77±8.91% and 42.83±8.91% for total sperm motility, and 7.30±8.74%, 24.87±8.74% and 29.70±8.74% for progressive sperm motility to groups CT, SPERM and FERT, respectively (P < 0.05).

Similar great results were observed on velocity parameters, with 69.87±10.64 µm/s, 85.43±10.64 µm/s and 89.47±10.64 µm/s for VAP, 52.47±9.34 µm/s, 65.87±9.34 µm/s and 72.60±9.34 µm/s for VSL, and 136.80±20.04 µm/s, 159.97±20.04 µm/s and 166.23±20.04 µm/s for VCL to groups CT, SPERM and FERT, respectively.

An improvement was observed even in plasma membrane integrity of groups that received motility inductors, as follows: 11.47±8.99% for CT, 36.23±8.99% for SPERM and 39.67±8.99% for FERT. No significant differences were observed between SPERM and FERT results, both showed good results on the parameters above.

Rapid sperm was the unique parameter evaluated that did not show significant difference between results of the groups evaluated (6.04±0.92% for CT, 6.74±0.92% for SPERM and 6.93±0.92% for FERT).

As occurs with other species, some factors can be detrimental to sperm motility and viability during frozen process. So, incubation with these motility stimulants is a good alternative to increment buffalo semen parameters, and avoid low quality of semen for artificial insemination (Monteiro et al., 2012; 2011; Melo et al., 2010).

In conclusion, diluted semen incubation with Sperm talp or Fert talp increases the quality of cauda epididymal sperm of buffalo bulls.

REFERENCES


Table 1. Parameters of post-thawed sperm samples recovered from the epididymidal cauda adding Sperm talp, Fert talp or without stimulants (control).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Motilities</th>
<th>Velocities</th>
<th>Rapid cell</th>
<th>Membrane integrity</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>MT</td>
<td>MP</td>
<td>VAP</td>
<td>VSL</td>
</tr>
<tr>
<td>CT</td>
<td>13.63±8.91b</td>
<td>7.30±8.74b</td>
<td>69.87±10.64b</td>
<td>52.47±9.34b</td>
</tr>
<tr>
<td>SPERM</td>
<td>38.77±8.91a</td>
<td>24.87±8.74a</td>
<td>85.43±10.64a</td>
<td>65.87±9.34a</td>
</tr>
<tr>
<td>FERT</td>
<td>42.83±8.91a</td>
<td>29.70±8.74a</td>
<td>89.47±10.64a</td>
<td>72.60±9.34a</td>
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