Conceptions Rates using Brahman Bull Semen Frozen in Milk Based Extender Containing Egg Yolk or Soybean Lipids: Field study in tropical conditions

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Introduction

• For years egg yolk have been used in the cryopreservation of semen from domestic and wild species as an effective component of cryopreservation media. However, the potential transmission of Salmonella species, the Newcastle Disease virus and the presence cryoprotective antagonists that inhibit respiration of spermatozoa and reduce their motility provides a strong argument for the elimination of egg yolk from semen cryopreservation procedures.

• It is assumed that the protective action of egg yolk to be due to small multilamellar vesicle of low density lipoproteins (LDL) that can be produced by centrifugation of egg yolk. Low density lipoproteins make up about two-thirds of the total solids of chicken egg yolk and are present in the soluble fraction of egg yolk. Phospholipids play an essential role in the stability of the LDL structure.

• Chemical comparison of chicken egg yolk composition and bull sperm membrane indicates that the common component present in high concentration in both the chicken egg yolk and the bull sperm membranes is phospholipid - phosphatidyl choline.
Objectives

• To examine the efficacy of double centrifuged chicken egg yolk in Brahman bull semen extender.

• To examine whether phosphatidyl choline of soybean-origin can substitute for the egg yolk in Brahman bull semen extender.
Environment and animals

• **Environmental conditions**

Semen was collected and AI was performed during the dry season between December and April in a tropical forest environment. The mean temperature for the region was between 26-30°C, with mean rainfall of 900-1500 mm/year and the relative humidity of 60-70%.

• **Animals**

  3 pure breed Brahman bulls
  174 cross breed Brahman cows
Semen extenders

- Basic extender solution was prepared in 1% milk containing 10 mg mL\(^{-1}\) of fructose and was supplemented by:

  - Extender 1* - (control), 8% of whole egg yolk
  - Extender 2* - 8% of rectified egg yolk
  - Extender 3* - 7.3 mg mL\(^{-1}\) of lipids of soybean-origin containing 10% of phosphatidyl choline

  *1000 IU of penicillin, 1mg mL\(^{-1}\) streptomycin and 150µg mL\(^{-1}\) lincomycin were added to each extender

- Glycerol was added to each extender in 2 steps in 15 minute intervals to a final glycerol concentration of 7%.
Materials and Methods
Semen collection, freezing and thawing

- Semen was collected by means of artificial vagina.
- Ejaculates with at least 60% motility were diluted in 2-steps as follows:
  
  **Step 1**, each ejaculate was split at 3 even parts and diluted at 26°C with each of the extenders containing no glycerol.

  **Step 2**, 14% of glycerol was added in 15 minute intervals to a final glycerol concentration of 7%.

- Semen was aspirated into 0.5mL plastic straws (20 x 10^6 sperm/per straw).
- Semen was frozen 7 cm above liquid nitrogen (LN_2) for 8 min, and then plunged into LN_2. Straws were thawed in a water bath at 37°C for 30s.
Experiment I

- Total of 157 cows were inseminated with semen collected from 3 different Brahman bulls A, B and C and frozen in 3 different extenders supplemented with:
  - whole egg yolk (control) Extender 1
  - rectified egg yolk Extender 2
  - lipids of soybean-origin Extender 3

<table>
<thead>
<tr>
<th>Bull</th>
<th>Extender 1</th>
<th>Extender 2</th>
<th>Extender 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>n=19</td>
<td>n=20</td>
<td>n=22</td>
</tr>
<tr>
<td>B</td>
<td>n=20</td>
<td>n=20</td>
<td>n=20</td>
</tr>
<tr>
<td>C</td>
<td>n=20</td>
<td>n=15</td>
<td>n=24</td>
</tr>
</tbody>
</table>

- Pregnancy rates were determined by palpation 45 Days after AI.
Experiment II

• Total of 117 cows were inseminated with semen collected from bull B and frozen in 3 tested Extenders:

  - Extender 1 (control)  n = 37
  - Extender 2           n = 48
  - Extender 3           n = 39

Post-thaw semen viability were determined by pregnancy rates 45 Days after AI.
Results
Experiment I

Table 1. Conception rates of cross breed Brahman cows inseminated with 3 different Brahman bulls semen frozen in milk-glycerol based extenders supplemented with 2 different preparations of egg yolk or soybean lipids.

<table>
<thead>
<tr>
<th>Bull</th>
<th>Whole egg Yolk</th>
<th>Rectified egg Yolk</th>
<th>Soybean lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cows Ai n</td>
<td>Pregnant n (%)</td>
<td>Cows Ai n</td>
</tr>
<tr>
<td>A</td>
<td>19</td>
<td>10 (34.5)</td>
<td>30</td>
</tr>
<tr>
<td>B</td>
<td>20</td>
<td>9 (45.0)</td>
<td>30</td>
</tr>
<tr>
<td>C</td>
<td>27</td>
<td>8 (29.6)</td>
<td>30</td>
</tr>
</tbody>
</table>
Experiment II

Table 2. Pregnancy rates of cross breed Brahman cows inseminated with one Brahman bull semen frozen in milk-glycerol based extenders supplemented with 2 different preparations of egg yolk or soybean lipids.

<table>
<thead>
<tr>
<th>Extender supplement</th>
<th>Whole egg yolk</th>
<th>Rectified egg yolk</th>
<th>Soybean lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows AI n</td>
<td>Pregnant n (%)</td>
<td>Cows AI n</td>
<td>Pregnant n (%)</td>
</tr>
<tr>
<td>37</td>
<td>(24.3)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48</td>
<td>(41.6)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>ab</sup>- percentages between columns with values not in common differ (P < 0.05)
Conclusions

- Results suggest that rectified egg yolk by means of double centrifugation improved efficacy of Brahman bull semen extender.

- Lipids of soybean origin containing 10% of phosphatidyl choline can successfully replace egg yolk in Brahman bull semen extender and are equally effective as rectified egg.