EFFECTS OF ADDITION OF POWDERED EGG YOLK IN TRIS-BASED DILUENTS ON THE QUALITY OF BUFFALO SEMEN STORED AT 5°C

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Abstract

This experiment was designed to investigate the effects of addition of powdered egg yolk (PEY) in Tris-based diluents on the quality of buffalo semen stored at 5°C. Post-dilution progressive motility and viability of spermatozoa were assessed at D-0, D-2, dan Dy-4. The effects of adding powdered egg yolk (PEY) in Tris-based diluent on the progressive motility of swamp buffalo spermatozoa was found to be very significant (p<0.05), except the viability. Progressive motility and viability of spermatozoa diluted with semen diluents with the addition 2.5% PEY were higher (p<0.05) than that of 7.5% PEY. Progressive motility of spermatozoa in diluents containing 2.5% TKT remained similar to those containing 5.0% PEY and 10.0% fresh egg yolk (FEY). The progressive motility of spermatozoa at 5.0% PEY and 10.0% KTS also remained similar to 7.5% PEY. Progressive motility and viability of spermatozoa in all four semen diluents containing different levels of yolks decreased with increasing time of storage at 5°C. In conclusion, the addition of 2.5% PEY in Tris-based swamp buffalo semen diluent was superior to the traditional egg yolk-based fresh diluents in maintaining the quality of chilled semen.

Keywords: swamp buffalo, powdered egg yolk, fresh egg yolk, semen diluent, quality of spermatozoa.

1. Introduction

Diluents used for semen preservation provide suitable media to prolong the survival of spermatozoa. semen diluents generally consist of energy sources, buffers and the provision of
appropriate osmotic pressure. Semen diluents based on whole chicken egg yolk (WCEY) are conventionally used as a common ingredient in the preservation of frozen and chilled semen (Wall and Foote 1999) in most livestock species including buffaloes, possibly because of their availability is easily obtained (Andrabi , 2009).

Egg yolks serve to provide protection against cold shock, maintain sperm motility, reduce loss of acrosomal enzymes and maintain sperm mitochondrial membranes (Salamon and Maxwell, 1995). The protective effect given by egg yolk to spermatozoa is due to the presence of a low density lipoprotein fraction of egg yolk. At present, the addition of egg yolks to cryodiluents semen diluents varies in concentration from 1.5 to 50% (Salamon and Maxwell, 1995). Although beneficial for spermatozoa that are cryopreserved, these egg yolks are from poultry, and can carry potential risks to sperm cells, because they may contain certain microbiological agents, or other pollutants that can damage sperm quality (Gil et al., 2003).

Powdered egg yolk can be a safe alternative as a substitute to fresh egg yolk, because it undergoes pasteurization during its synthesis process (Landfeld et al., 2002). In practical reality, the collection of chicken egg yolk for use in the process of semen dilution is quite complicated, starting from screening the disease correctly, disinfection, the skill to completely breaking the egg shell and inner membrane to separate the yolk part from albumin and chalazae (Andrabi et al., 2008). In addition, it is also necessary to ultracentrifuge the egg yolk before using in semen extender (Ansari et al., 2010). The use of powdered egg yolk as a part of diluent for semen cryopreservation proved to be effective and significantly showed a higher quality of spermatozoa after frozen-thawed semen compared with fresh egg yolk during freezing of ram (Marco-Jime´nez et al., 2004), bull (Ansari et al., 2010), and Murrah buffalo semen (Singh et al., 2015; Kumar et al., 2016).

The study of using powdered egg yolk as a diluent for dilution of swamp buffalo semen stored at 5°C in NTB seems to have never been reported. Therefore, this study was conducted with the aim of investigating the effect of adding powdered egg yolk and fresh egg yolk in a Tris-based semen extender on the quality of spermatozoa stored at 5°C for 4 days through testing of motility and viability parameters.

2. Materials And Methods

2.1 Animals
Three adult and healthy male buffaloes, aged between 2.5 to 3 years with body weight around 350 kg to 380 kg maintained at Teaching Farm, Lingsar Village, Narmada Subdistrict were used in the study. The animals were kept under identical management and feeding conditions throughout the course of the study.

2.2 Semen collection
One ejaculate was collected with an artificial vagina (40-45°C) once a week for five months. Immediately after collection, semen volume, color, mass motility and individual motility were recorded. Ejaculates which had more than 70% progressive sperm motility, mass movement score +3, and milky white color were used in this study. The concentration of sperm in the semen sample was determined by hemocytometer method.

2.3 Preparation of diluent, dilution and storage of chilled semen
Four Tris-based semen diluents (Tris-citric acid-fructose-glycerol) each containing 2.5%, 5%, and 7.5% w/v powdered egg yolk (PEY) and 10% v/v fresh egg yolk (FEY) were prepared and stored in a water bath (32°C). Ejaculates with 70% progressive sperm motility were split into four parts. Part I, II, and III were diluted with Tris diluents each containing 2.5%, 5%, and 7.5% w/v PEY. Part 4 was diluted with Tris diluent containing 10% v/v FEY. After dilution, all semen samples were cooled in the refrigerator until the temperature drops from 32 to 5°C.

2.4 Assessment of diluted semen
Assessments of individual motility and viability of spermatozoa were carried out at intervals of D-0, D-2 and D-4d storage. A drop of diluted semen sample was placed on a pre-warmth glass slide (37°C) and covered with a cover glass, then the percentage of progressive motility of spermatozoa was subjectively assessed under the microscope (400x magnification). The percentage of live and dead spermatozoa was determined by differential staining technique using an eosin-nigrosine stain solution. Determination of the number of live spermatozoa (white sperm heads not stained) and dead spermatozoa (sperm heads stained red/pink) were carried out by counting 100 spermatozoa in a glass slide under a microscope 400x magnification.
2.5 Statistical Analysis

Data on parameters of progressive motility and viability of spermatozoa in the four semen diluents were statistically analyzed using the SPSS-IBM 22 program to calculate the analysis of variance (ANOVA) of these two spermatozoa parameters. Significant differences between mean values were calculated using Duncan multiple range test (DMRT) at a significance level of p<0.05.

3. Results

The results of assessment of progressive motility and viability of swamp buffalo spermatozoa diluted in various semen diluents containing powdered egg yolk (PEY) and fresh egg yolk (FEY) stored at 5°C for 4 days are shown in Table 1.

Table 1. Average (± S.D) progressive motility and viability of swamp buffalo spermatozoa during stored at 5°C in semen diluents containing a various levels of powdered egg yolk and fresh egg yolk

<table>
<thead>
<tr>
<th>Variables</th>
<th>Concentrations (%)</th>
<th>Powdered egg yolk</th>
<th>Fresh egg yolk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
<td>2.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Motility</td>
<td>0</td>
<td>77,73±3,</td>
<td>77,27±3,</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>44</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>70,91±6,</td>
<td>67,27±9,</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>84</td>
<td>.14</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>38,18±22</td>
<td>30,91±20</td>
</tr>
<tr>
<td></td>
<td>,72</td>
<td>,59</td>
<td>,28</td>
</tr>
<tr>
<td>Viability</td>
<td>0</td>
<td>96,64±1,</td>
<td>95,27±1,</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>68</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>93,18±3,</td>
<td>92,09±3,</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>91</td>
<td>96</td>
</tr>
</tbody>
</table>
Progressive motility (PM) of swamp buffalo spermatozoa in semen diluted with the addition of 2.5% PEY was significantly different (p<0.05) with that received 7.5% PEY. However, PM spermatozoa in diluent containing 2.5% PEY was not significantly different (p<0.05) with those containing 5.0% PEY and 10.0% FEY. The differences in PM between spermatozoa diluted in 5.0% PEY and 10.0% FEY with 7.5% PEY was also not significant (p>0.05).

Progressive motility of spermatozoa in semen diluents containing a various levels of PEY and FEY diluents showed a significant decrease (p>0.05) between D-0 and D-2 and D-4 storage at 5ºC. PM of swamp buffalo spermatozoa in all four diluents was more than 60% on D-2 of storage, but PM spermatozoa in semen diluents containing 2.5% PEY was higher (70.91%) than 7.5% PEY (61.82%). PM spermatozoa on D-4 decreased dramatically in all diluents to reach level of less than 40% (Table 1).

Viability of swamp buffalo spermatozoa preserved at 5ºC for 4 days was not significantly different (p>0.05) among the four semen diluents containing a various levels of PEY and FEY. However, the viability of spermatozoa decreased significantly (p<0.05) during chilled storage (5ºC). Viability of spermatozoa up to D-2 of storage was found to be above 90%, thereafter decreased slightly to reach level of less than 80% on D-4 (Table 1).

4. Discussions

The use of fresh egg yolks as the main component of semen diluents has been carried out in the preservation of most mammalian semen such as cattle, buffalo, goat, sheep, horse, pig, and even human. This study aims to investigate the effect of addition of PEY in Tris-based semen diluents on the quality and the storeability of swampu buffalo spermatozoa at 5ºC for 4 days.

Egg yolks contain the cryoprotectant fraction which is associated with a low density lipoprotein called lecithin which can prevent membrane damage to spermatozoa. In this study, the addition of 2.5% PEY in Tris-based diluents was able to maintain PM spermatozoa better than that of 7.5% FEY. The 2.5% concentration of PEY is equivalent in its ability to maintain PM spermatozoa compared to 5.0% PEY and 10% FEY. This shows that 2.5% PEY
is the ideal concentration compared to the other three concentrations of semen dilution tested. This result is different from the results of study on the use of PEY as reported by Singh et al. (2015), who found the best level is 5% PEY for cryopreservation of buffalo semen. On the other hand, Kumar et al. (2016) found that 4% PEY was the best level for frozen-thawed buffalo semen.

However, Ansari et al. (2010) when added 20% PEY to Tris-based diluents recorded the average percentage of PM buffalo spermatozoa of 68.3±3.44% post dilution, which was slightly lower than the present study with the average PM of 77.73±3.44% on D-0 or post dilution. Similarly, it was lower compared to PM buffalo spermatozoa recorded by Kumar et al. (2016), who accounted to be 74.67±3.44% post-dilution. In the present study the gradual decreases in PM swamp buffalo spermatozoa in semen diluted with different diluents during the storage period supports the results of previous studies (Gundogan et al., 2003).

5. Conclusions
The addition of powdered egg yolk as much as 2.5% in Tris-based semen diluents is significantly affected progressive motility of buffalo spermatozoa compared to fresh egg yolk with a level of 10% fresh egg yolk and other powdered egg yolk concentrations (5.0% and 7.5%). Although statistically, the effect of addition 2.5% powdered egg yolk semen diluent was not significant for spermatozoal viability, numerically, the 2.5% level of PEY showed a potency in maintaining the viability of semen during chilled storage of swamp buffalo spermatozoa.

Tris-based buffalo semen diluents prepared with the addition of 2.5% PEY (v/v) is superior to the traditional fresh egg yolk-based diluent (FEY) in maintaining the quality of buffalo bulls semen preserved in chilled form. Therefore, it is recommended that PEY be used as an alternative to fresh egg yolk-based diluents for the liquid preservation of swamp buffalo diluents for short storage periods.

6. Acknowledgements
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7. Reference


