Quality of Labrador Retriever dog semen on short-term preservation in different extenders

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ABSTRACT

The objective of the present study was to find the comparative efficacy of three extenders to preserve semen of Labrador-Retriever (LR) dogs at 5°C for a short term. The semen samples of LR dogs were collected by digital manipulation method and extended at the rate of 1:4 in Tris-Egg Yolk- Citric Acid-Glucose (TEYCAG), Tris-Egg Yolk- Citric Acid-Fructose (TEYCAF) and Egg Yolk-Citrate-Glycine-Glucose (EYCGG) extenders by split sample technique. Semen was evaluated at 0, 24, 48, 72, 96 and 120 hours of preservation. Mean motile, live and HOST-reacted sperm and acrosomal, head, mid piece and tail abnormalities of spermatozoa varied significantly (P<0.01) between extenders and between preservation periods. The interactions between extender and preservation period were also significant (P<0.01) except for HOST-reacted and head abnormalities of sperm. The highest mean motile, live and HOST-reacted sperm were recorded in TEYCAG extender which did not differ significantly from that of TEYCAF extender. Mean per cent sperm acrosomal and tail abnormalities were significantly (P<0.05) lower, and the incidences of mean sperm head and mid piece abnormalities were also lower in TEYCAG, but not significantly from that in TEYCAF irrespective of hour of preservation. Per cent motile, live and HOST-reacted sperm were significantly (P<0.05) lower and sperm acrosomal, head, mid piece and tail abnormalities were significantly (P<0.05) higher in EYCGG as compared to that in TEYCAG and TEYCAF irrespective of hour of preservation. It was concluded that the semen of LR dog sustained good quality during preservation up to 5 days at 5°C suitable for successful artificial insemination and would be preserved better in TEYCAG and TEYCAF extenders than in EYCGG extender, since more than 50 per cent sperm motility and live sperm were maintained up to 120 hours of preservation in the former two extenders.

Key words: Abnormalities, Acrosome, HOST, Labrador-Retriever dog, Livability, Sperm motility.

INTRODUCTION

Dog as a pet animal has high sense of smell which enables them to perform different incredible works. The demand of superior quality dogs for breeding has accelerated during recent times. The non-availability of the male of desired breed in a locality and the possibility of obtaining superior germ-plasm of selected dogs elsewhere have aroused interest among the dog breeders to adopt artificial insemination (A.I.) in bitches. Short term storage of semen is highly desirable to reduce the transportation cost and to inseminate bitches more than once for successful impregnation as they have comparatively longer receptive phase in their estrous cycle. A minimum standard in the quality of semen should be maintained during preservation for successful A.I. The quality of semen preserved in an extender can be assessed in vitro based on sperm motility, live sperm, HOST-reacted sperm and abnormality of acrosome and other sperm components. Evaluations of sperm parameters during preservation of semen of a few breeds of dog and their admixture were carried out earlier (Hermansson and Linde Forsberg, 2006; Varela Junior et al., 2009; Hori et al., 2014). However, there is no available literature on seminal quality during preservation of LR dog reared under refrigeration condition. Hence the present work was taken up to find the comparative efficacy of three extenders to retain the quality of LR dog semen during preservation at 5°C.

MATERIALS AND METHODS

Animals, locations and management: Three Labrador Retriever dogs reared singly by well-off individual owners residing in different locations of Guwahati city were used in the present study. The dogs were between 2½ and 3½ years of age. They were thoroughly examined for general and reproductive health before inclusion in the study and were found to be clinically healthy.

Semen collection and evaluation: A total of 15 ejaculates, five from each of three LR dogs, were collected at weekly intervals by digital manipulation method. The first and second fractions of the ejaculates were collected combinedly in a glass graduated collection cup for evaluation while the third fraction was collected in a separate cup for sexual

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satisfaction of the male. Immediately after collection, the semen sample was split into three equal fractions and kept in three glass graduated collection tubes that had a mean concentration of 440.24 ± 16.20 million sperms per ml. The fractions were extended (1:4) separately in Tris-Egg Yolk-Citric Acid-Glucose (TEYCAF) (Verstegen et al., 2005), Tris-Egg Yolk- Citric Acid-Fructose (TEYCAF) (Foote, 1970) and Egg Yolk- Citrate-Glycine-Glucose (EYCGG) (Foote and Leonard, 1964) extenders at 35°C. The tubes containing the extended semen were gently shaken in between the palms for homogenization and gradually cooled to 5°C in a refrigerator for preservation.

Sperm motility and live sperm were evaluated at 0 hour (i.e., immediately after extension) till 120 hours of preservation at 24 hours interval using standard methods. Acrosomal abnormalities were studied using Giemsa stain and sperm head and mid piece abnormalities were counted in semen smears stained with William’s stain under compound microscope and tail abnormalities were recorded in buffered formal saline solution under phase contrast microscope and expressed in percentages. The preserved semen was evaluated 0 hour (i.e., immediately after extension) through 120 hours of preservation at 24 hours interval. The extended semen samples preserved at 5°C for 24, 48, 72, 96 and 120 hours were subjected to hypo-osmotic solution at room temperature. The functional integrity of the sperm membrane was studied by using a Hypo-Osmotic solution as per the method described by Jeyendran et al. (1984).

The animals were maintained following the norms required under Institutional Animal Ethics Committee. Statistical analysis: Statistical analysis of the data was done following standard methods (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

The mean sperm motility, live sperm, acrosome, head, mid piece and tail abnormalities preserved in the three extenders at 5°C for different hours are presented in Table 1 and that for HOST–reacted sperm are presented in Table 2.

The mean sperm motility and live sperm differed significantly (P<0.01) between extenders, between preservation periods and due to their interactions. The mean percentage of sperm motility although did not differ significantly between TEYCAF and EYCGG extenders at different periods of preservation, it was significantly (P<0.05) lower in EYCGG as compared to that in TEYCAF extender at 48 hours of preservation. It was also significantly (P<0.05) lower in EYCGG extender than that in TEYCAF and

### Table 1: Per cent sperm motility, live sperm and abnormalities of sperm components (Mean± SE) in Labrador-Retriever dog during preservation (5°C) in different extenders.

<table>
<thead>
<tr>
<th>Sperm Parameter</th>
<th>Extender</th>
<th>0</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm motility</td>
<td>TEYCAF</td>
<td>94.69±0.85</td>
<td>81.56±0.23</td>
<td>76.56±1.97</td>
<td>70.31±0.22</td>
<td>63.75±0.22</td>
<td>73.65±1.59</td>
<td></td>
</tr>
<tr>
<td>EYCGG</td>
<td>94.69±0.85</td>
<td>80.94±0.05</td>
<td>74.69±2.26</td>
<td>68.13±0.62</td>
<td>61.25±0.35</td>
<td>72.19±1.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>94.69±13.66</td>
<td>79.69±11.50</td>
<td>73.13±10.55</td>
<td>65.52±9.45</td>
<td>58.33±8.42</td>
<td>71.05±9.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live sperm</td>
<td>TEYCAF</td>
<td>98.44±0.26</td>
<td>91.62±1.23</td>
<td>86.26±1.25</td>
<td>81.73±1.51</td>
<td>73.82±2.15</td>
<td>66.76±1.28</td>
<td></td>
</tr>
<tr>
<td>EYCGG</td>
<td>98.44±0.26</td>
<td>91.14±1.28</td>
<td>84.83±1.24</td>
<td>79.81±1.60</td>
<td>71.45±2.35</td>
<td>64.97±2.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>98.44±0.15</td>
<td>89.64±0.79</td>
<td>83.69±0.95</td>
<td>77.70±1.19</td>
<td>69.20±1.54</td>
<td>61.56±1.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acrosome</td>
<td>TEYCAF</td>
<td>0.43±0.20</td>
<td>1.83±0.32</td>
<td>3.40±0.52</td>
<td>6.30±0.52</td>
<td>9.20±0.73</td>
<td>13.03±0.82</td>
<td></td>
</tr>
<tr>
<td>EYCGG</td>
<td>0.83±0.30</td>
<td>2.47±0.44</td>
<td>4.63±0.58</td>
<td>7.50±0.46</td>
<td>10.60±0.81</td>
<td>14.60±1.05</td>
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<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.87±0.16</td>
<td>2.66±0.27</td>
<td>4.88±0.38</td>
<td>7.88±0.34</td>
<td>11.05±0.49</td>
<td>15.64±0.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head</td>
<td>TEYCAF</td>
<td>0.87±0.22</td>
<td>1.57±0.27</td>
<td>1.80±0.25</td>
<td>2.03±0.25</td>
<td>2.10±0.25</td>
<td>2.15±0.11</td>
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</tr>
<tr>
<td>EYCGG</td>
<td>0.77±0.28</td>
<td>1.20±0.39</td>
<td>2.30±0.38</td>
<td>2.57±0.40</td>
<td>2.77±0.37</td>
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<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.98±0.19</td>
<td>1.52±0.24</td>
<td>2.10±0.22</td>
<td>2.63±0.24</td>
<td>2.78±0.25</td>
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<td></td>
</tr>
<tr>
<td>Mid piece</td>
<td>TEYCAF</td>
<td>0.20±0.14</td>
<td>0.57±0.18</td>
<td>0.63±0.17</td>
<td>0.93±0.24</td>
<td>1.53±0.25</td>
<td>1.70±0.24</td>
<td></td>
</tr>
<tr>
<td>EYCGG</td>
<td>0.30±0.19</td>
<td>0.50±0.22</td>
<td>0.67±0.24</td>
<td>1.23±0.24</td>
<td>1.87±0.23</td>
<td>2.00±0.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.30±0.11</td>
<td>0.67±0.16</td>
<td>1.43±0.21</td>
<td>2.27±0.33</td>
<td>3.03±0.27</td>
<td>3.43±0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail</td>
<td>TEYCAF</td>
<td>1.47±0.38</td>
<td>5.47±0.46</td>
<td>8.43±0.44</td>
<td>10.83±0.49</td>
<td>12.77±0.43</td>
<td>14.53±0.36</td>
<td></td>
</tr>
<tr>
<td>EYCGG</td>
<td>1.87±0.39</td>
<td>6.07±0.43</td>
<td>8.83±0.50</td>
<td>11.53±0.45</td>
<td>13.67±0.46</td>
<td>15.43±0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>2.07±0.25</td>
<td>6.36±0.28</td>
<td>9.59±0.33</td>
<td>12.43±0.37</td>
<td>14.87±0.44</td>
<td>17.24±0.57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

15 ejaculates

Means having at least one common letter superscript in a column and a row within a parameter do not differ significantly.

A, B, C, D, E, F: Means bearing different superscripts in a column and a row within a parameter differ significantly (P<0.05).
TEYCAF extenders at 72, 96 and 120 hours and irrespective of hour of preservation. This could be ascribed to shift in osmolarity in EYCGG to which dog spermatozoa are sensitive (Jongsasen et al., 2002). Although not significant, the mean percentage of motile sperm was higher in TEYCAG than in TEYCAF during preservation. This could be due to preferential utilization of glucose molecule rather than fructose by canine spermatozoa (Ponglowhapan et al., 2004) which might be attributed to higher capacity of the glucose transport system in dog sperm (Rigau et al., 2002). The mean sperm motility was maintained at >50 per cent in TEYCAG and TEYCAF extenders up to 120 hours of preservation at 5°C, while EYCGG extender could sustain 50 per cent sperm motility till 96 hours of preservation. The percentage of mean live sperm although did not differ significantly between TEYCAG and TEYCAF extenders at different hours of preservation, it was significantly (P<0.05) lower in EYCGG as compared to that in TEYCAG and TEYCAF extenders irrespective of hour of preservation which could be indicative of least suitability of EYCGG extender in storing LR dog semen under refrigeration condition.

The mean percentage of sperm motility and live sperm decreased significantly (P<0.05) at 24 hours of preservation as compared to that immediately after extension in all the three extenders. Thereafter while the sperm motility was found to fall significantly (P<0.05) between each successive hour of preservation from 24 to 120 hours in EYCGG, it decreased significantly (P<0.05) only from 96 to 120 hours of storage in both TEYCAG and TEYCAF. This could be attributed to difference in maintaining the osmotic pressure and pH of extended semen during storage between the extenders (Tatsuji et al., 2003). The mean percentage of live sperm decreased significantly (P<0.05) between each successive period of preservation till 120 hours in all the extenders except between 48 and 72 hours of preservation in TEYCAF which could be indicative of its superior efficacy. Sperm motility and live sperm also declined significantly (P<0.05) with increase in period of preservation irrespective of extender. This could be ascribed to changes in spermatozoa due to senescence with advancement in time under storage condition.

There was significant (P<0.01) difference in mean acrosomal abnormalities between different extenders, between preservation periods and due to interactions (P<0.05). This indicated that the main effects were not independent. The mean percentage of sperm acrosomal abnormalities did not differ significantly between TEYCAG and TEYCAF extenders at all hours of preservation. But it was significantly (P<0.05) higher in EYCGG extender from that of TEYCAG extender at 24 hours of preservation. It was also significantly (P<0.05) higher as compared to that of TEYCAG and TEYCAF extenders during preservation from 48 to 120 hours of preservation. This was indicative of lower efficacy of EYCGG extender in protecting sperm acrosome during preservation as compared to TEYCAG and TEYCAF extenders. It was observed in the present study that the mean percentage of acrosomal abnormalities differed significantly (P<0.05) between the three extenders irrespective of preservation period, the magnitude being the lowest in TEYCAG extender, which increased successively in TEYCAF and EYCGG extenders. This could be due to difference in the capacity of the extenders to protect the acrosome of spermatozoa from detrimental effects during the cooling process and subsequent cold storage at 5°C for prolonged period up to 120 hours. A cooling-induced membrane transitions in the acrosomal membrane has been incriminated in increasing acrosomal damage of spermatozoa (Parks and Graham, 1992). Conferment of better acrosomal integrity by TEYCAG extender in comparison with TEYCAF and EYCGG extenders could be due to its capacity to prevent the false acrosome reaction associated with sperm death or irreversible damage (Rota et al., 1995).

The mean percentage of acrosomal abnormalities increased significantly (P<0.05) between each successive period of preservation from 48 to 120 hours in TEYCAG, from 24 to 120 hours in TEYCAF, and from 0 to 120 hours in EYCGG extender. This was indicative of comparative superiority of TEYCAG over TEYCAF, and that of TEYCAF over EYCGG extender in providing protection and maintenance of integrity of acrosome of dog spermatozoa. The mean percentage of acrosomal abnormalities increased significantly (P<0.05) with increase in each period of observation during preservation irrespective of extender which was corroborated by the findings of Kadirvel and Sreekumaran (2003). Cellular integrity and permeability might be altered during dilution, cooling, storage and ageing which could lower the acrosomal integrity and increase the proportion of acrosomal abnormalities with prolongation of

### Table 2: Incidence (%) of HOST-reacted Labrador-Retriever dog spermatozoa (Mean ± S.E.) during preservation (5°C) in different extenders.

<table>
<thead>
<tr>
<th>Extender</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
<th>120</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEYCAG</td>
<td>93.44±1.92</td>
<td>87.99±4.35</td>
<td>85.03±4.23</td>
<td>82.20±4.42</td>
<td>79.51±4.25</td>
<td>85.63±1.87</td>
</tr>
<tr>
<td>TEYCAF</td>
<td>92.20±1.72</td>
<td>85.56±3.82</td>
<td>82.25±4.32</td>
<td>78.75±5.17</td>
<td>70.00±3.63</td>
<td>81.75±2.12</td>
</tr>
<tr>
<td>EYCGG</td>
<td>85.65±3.01</td>
<td>75.34±6.86</td>
<td>68.88±7.46</td>
<td>59.33±5.10</td>
<td>52.50±3.71</td>
<td>68.34±3.15</td>
</tr>
<tr>
<td>OVERALL</td>
<td>90.43±1.49</td>
<td>82.97±3.11</td>
<td>78.72±3.46</td>
<td>73.43±3.61</td>
<td>67.34±3.43</td>
<td></td>
</tr>
</tbody>
</table>

Means having at least one letter superscript in common within column and within row do not differ significantly.
preservation period by 24 hours. Gradual increase in the proportion of acrosomal abnormalities with increase in period of preservation also could be due to peroxidation effect (Jones and Mann, 1977; Pursel, 1979). It was observed that there was increase in phospholipids and cholesterol in the seminal plasma on storage, and high concentrations of these plasmatic components were reported to cause destructive changes in sperm membranes (Dimitrov et al., 2009). This might explain the obtained higher percentage of acrosomal abnormalities in spermatozoa with increase in duration of preservation.

There was significant (P<0.01) difference in mean sperm head abnormalities between extenders and between preservation periods, but no significant difference was observed in the interaction between extender and preservation period. This indicated that the main effects were independent. The mean incidence of sperm head abnormalities was significantly (P<0.05) higher in EYCGG extender as compared to that in TEYCAG and TEYCAF extenders irrespective of hour of preservation. This might indicate that the latter two extenders provided better protective action against changes in spermatozoan head integrity than the former mentioned extender during preservation at 5°C. In the present study sperm head abnormalities included tailless heads or free normal heads which was observed under phase contrast microscope after preserving the semen samples in formal saline solution. The significant increase in sperm head abnormalities in EYCGG extender could be due to higher incidence of free normal heads in it as compared to TEYCAG and TEYCAF extenders. Impaired biochemical milieu during preservation of dog semen in EYCGG extender could have contributed to accelerate separation of spermatozoan head from the tail at the vulnerable neck region of normal spermatozoa giving rise to elevated occurrence of free normal sperm heads in it. However, the incidence of sperm head abnormalities in EYCGG extender was not ostensibly high and it revolved around a narrow range in the three extenders during preservation.

Although the mean sperm head abnormalities differed significantly between preservation periods, it did not differ significantly in the present study between each successive period of preservation irrespective of extender. The incidences were only significantly (P<0.05) higher during 48 to 120 hours of preservation as compared to that at 0 hour of preservation, during 72 to 120 hours of preservation in comparison with that at 24 hours of storage, and at 96 and 120 hours of preservation than that of 48 hours-kept semen irrespective of extender. The findings might suggest that the incidence of sperm head abnormalities increased only on prolongation of preservation with deterioration in keeping quality of the extenders.

It was observed that there was significant (P<0.01) difference in mean sperm mid piece abnormalities between extenders, between preservation periods and due to interactions. This indicated that the main effects were not independent. The mean incidence of sperm mid piece abnormalities did not differ significantly between TEYCAG, TEYCAF and EYCGG extenders at 0 and 24 hours of preservation. It did not differ significantly between TEYCAG and TEYCAF extenders during 48 to 120 hours of preservation, while it was significantly (P<0.05) higher in EYCGG extender as compared to that in TEYCAG and TEYCAF extenders during the corresponding period of storage. Tsutsui et al. (2003) also observed increase in the incidence of abnormalities of mid piece of canine sperm on preservation for 8 days at 4°C in egg yolk-tris-fructose-citrate solution. It was also significantly (P<0.05) higher in EYCGG extender than in TEYCAG and TEYCAF extenders irrespective of period of preservation. This might reflect differences in osmolarity of the extenders used during prolonged period of storage. It was found that the incidence of mid piece abnormalities did not differ significantly between successive periods of preservation in TEYCAG extender, while it was significantly (P<0.05) higher at 96 hours than at 72 hours of preservation in TEYCAF extender. This could suggest that TEYCAG extender was more suitable in preserving canine semen in regard to maintenance of sperm mid piece integrity. In EYCGG extender, the incidence of mid piece abnormalities rose significantly (P<0.05) during 24 to 96 hours of preservation at every consecutive period of observation from the preceding period. It could imply concomitant increase in mid piece abnormalities with reduced efficacy of EYCGG extender with increase in period of storage. The significant (P<0.05) increase in the percentage of mid piece abnormalities during 48 to 96 hours of preservation irrespective of extender could indicate progressive increase in mid piece abnormalities with increase in period of preservation as the inefficacy of the extenders intensified.

The mean incidence of sperm tail abnormalities did not differ significantly between TEYCAG and TEYCAF extenders, however, it was significantly (P<0.05) higher in EYCGG as compared to that in TEYCAG extender at 0 hour of preservation. This could indicate that changes in sperm plasma membrane was more pronounced with the addition of EYCGG extender due to changes in osmotic pressure in semen giving rise to significantly (P<0.05) higher sperm tail abnormalities. It was found that there was significant (P<0.01) difference in mean sperm tail abnormalities between extenders, between preservation periods and due to interactions. This indicated that the main effects were not independent. It was recorded that the mean percentage of sperm tail abnormalities did not differ significantly between
TEYCAG and TEYCAF extenders at all periods of observations during preservation. It was significantly (P<0.05) higher in EYCGG extender than that in TEYCAG and TEYCAF extenders at each period of preservation at 5°C from 24 to 120 hours. This could be ascribed to diminished efficacy of EYCGG extender in protecting the dog sperm plasmalemma from cold exposure during the cooling process and subsequent storage under chilled condition that resulted in significantly higher incidence of tail abnormalities in this extender. Bateman (2001) reported that when canine semen was slowly cooled to 0°C, a significantly greater proportion of spermatozoa had bent tail tips as compared to fresh semen. The efficacy of TEYCAG and TEYCAF extenders was revealed from the present findings that the percentage of mean sperm tail abnormalities at 120 hours of preservation was significantly (P<0.05) lower in the two extenders than that in EYCGG extender.

The incidence of mean sperm tail abnormalities was found to differ significantly (P<0.05) between TEYCAG, TEYCAF and EYCGG extenders irrespective of period of preservation, the incidence being the lowest in TEYCAG that increased successively in TEYCAF and EYCGG extenders. Exposure of dog semen to ultra-cooled condition was known to increase its concentration of sodium while decreasing that of potassium (Quinn and White, 1967). Changes in sodium and chlorine concentrations that are the principal cation and anion present in dog seminal plasma (Hafez, 1970) might be contemplated to influence the ability of spermatozoan membrane to protect the sperm from undergoing changes in tail configuration in different extenders. This might explain the significant difference in the incidence of tail abnormalities in different extenders irrespective of period of preservation. Significant increase in the mean incidence of tail abnormalities with increase in the period of storage at 5°C irrespective of extenders obtained in this study might reflect reduction in the efficacy of extenders in protecting the sperm flagellar membrane with progress in time. Tsutsumi et al. (2003) also reported increase in the incidence of tail abnormalities of canine sperm on preservation for 8 days at 4°C in egg-yolk-tris-fructose-citrate solution.

In the present study it was observed that there was significant (P<0.01) difference in mean HOST-reacted sperm between extenders and between preservation periods. However, the interaction between extender and preservation period was not statistically significant. This indicated that the main effects were independent. In the present study the percentage of HOST-reacted sperm did not differ significantly between TEYCAG, TEYCAF and EYCGG extenders at all hours of preservation. Although non-significant, the percentage of HOST-reacted sperm was higher in TEYCAG than in TEYCAF extender at 120 hours of preservation. Per cent HOST-reacted spermatozoa did not differ significantly between TEYCAG and TEYCAF extenders irrespective of preservation period. This might suggest that the two extenders were equally efficacious in providing functional integrity of the plasma membrane of spermatozoa during preservation. However, the percentage of HOST-reacted sperm was somewhat higher in TEYCAG than that in TEYCAF extender during 24 to 96 hours of preservation. The mean percentage of HOST-reacted sperm was significantly (P<0.05) lower in EYCGG extender as compared to that in TEYCAG and TEYCAF extenders irrespective of hour of preservation. This might indicate that the percentage of spermatozoa with an intact membrane decreased in EYCGG extender consequential to its impaired protection of spermatozoan membrane. Differential action of the extenders in shielding sperm membrane in dog semen exposed to hypo-osmotic solution has been documented. Rota et al. (1995) reported that integrity of plasma membrane of spermatozoa was better maintained in Egg yolk-Tris extender than in Egg yolk-milk and Egg yolk-cream extender when dog semen preserved at 4°C was subjected to hypo-osmotic swelling test.

The incidence of HOST-reacted sperm during 48 to 120 hours of preservation decreased significantly (P<0.05) from that at 24 hours of preservation irrespective of extender. This suggested that the integrity of plasma membrane and functional intactness of spermatozoa decreased with increase in preservation period. Rota et al. (1995) also recorded that the percentage of swollen canine sperm i.e., with an intact membrane decreased over time when dog semen preserved in Egg yolk-Tris, Egg yolk-milk and Egg yolk-cream extender at 4°C underwent hypo-osmotic swelling test. It was revealed in the present investigation that although the HOST-reacted spermatozoa differed significantly between preservation periods, it did not differ significantly between each successive hour of observation during preservation at 5°C irrespective of extender. The percentage of HOST-reacted sperm was significantly (P<0.05) lower at 96 and 120 hours of storage as compared to that at 48 hours of preservation and it was also significantly (P<0.05) lower at 120 hours of preservation than that at 72 hours of storage. This might imply that the decline in percentage of HOST-reacted sperm during preservation was not abrupt but rather gradual and the reaction of sperm plasma membrane to hypo-osmotic solution diminished gradually with prolongation of preservation period.

CONCLUSION

LR dog semen could be better preserved in TEYCAG and TEYCAF extenders at 5°C since percentages of sperm motility, live sperm, HOST-reacted sperm were significantly higher and that of abnormalities of acrosome, head, mid piece and tail of spermatozoa were significantly lower as compared to EYCGG extender during preservation of extended semen for 120 hours.
REFERENCES


