EFFECT OF SEMEN DILUENT ADDITIVES ON SPERMATOZOAL VIABILITY OF KANKREJ BULL SEMEN FOLLOWING CRYOPRESERVATION

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Total 60 ejaculates, 10 each from 6 mature Kankrej bulls were divided into 3 equal aliquots, the first aliquot was added with TFYG diluent with EDTA (0.1%), second with TFYG diluent and caffeine (0.5%) as semen diluent additives and third without additive as control and each aliquot was evaluated at post diluted, post equilibrated and post thawed stages for individual motility, live sperm count, abnormal sperm count and acrosomal integrity. The percentage of individual motility, live sperm and acrosomal integrity were significantly higher (P < 0.05) and abnormal sperm count was significantly lower (P < 0.05) in presence of EDTA and caffeine as compared to TFYG diluent without any additive at all stages of semen preservation. It can be concluded that motility, viability and acrosomal integrity of spermatozoa were increased and abnormality was decreased after addition of EDTA and caffeine as additives when compared with TFYG diluent without any additive.

Key words: Kankrej bull, Semen additives, Cryopreservation, Spermatozoal Morphology

Kankrej cattle has been important dual purpose breed of India. They have been well adopted in North Gujarat and have proved to be superior to crossbreds with respect to milk production and disease resistance (Annual progress Report-2009, LRS, SDAU, Sardarkrushinagar, Gujarat). Motility, live sperm count, abnormality and acrosomal integrity could be a practical utility in routine semen evaluation to predict keeping quality and freezability of Kankrej bull semen. The literature on the beneficial effect of extender additive on the keeping quality and freezability of bovine semen is quite large (Kumar et al., 2001). Moreover, Rana and Dhami (2003) have reported the interrelationship of various spermatozoal attributes at the initial, post thawed and post refrigeration stages after Sephadex filtration of crossbred and Gir bull semen. But reports on such interrelationship for spermatozoal attributes at different steps of semen processing and preservation with the use of additive are meagre. Hence, it was proposed to take up the present study to preserve and assess the fertility of the semen by pre and post thaw motility, viability and acrosomal integrity of spermatozoa by using commercial semen additives.

MATERIALS AND METHODS
Total six Kankrej bulls, ranging from 3 to 41/2 years of age and clinically normal, were selected as semen donors from Dama semen production Unit, Banas dairy, Palanpur. All the standard procedures from semen collection to its storage in nitrogen were followed in strict aseptic condition. A total of ten ejaculates were obtained from each bull for ten weeks. Each ejaculates were divided in three equal aliquots. Aliquot-1 was diluted with TFYG diluent added with EDTA (0.1%), aliquot-2 was diluted with TFYG diluent added with caffeine (0.5%) and aliquot-3 was diluted with TFYG diluent without any additive and served as control. The dilution rate was calculated keeping in view the sperm concentration per dose of diluted semen. Each aliquot of each group was evaluated at post diluted, post equilibrated and post thawed stages for individual motility, live sperm count, abnormal sperm count and acrosomal integrity.
The means and standard errors of all the traits were calculated using 180 observations (60 ejaculates × 3 aliquots) and used two factorial CRD at P < 0.05 level of significance. The analysis of variance was worked out as per the procedure described by Snedecor and Cochran (1994).

**RESULTS AND DISCUSSION**

Overall mean values of individual motility and viability of spermatozoa was significantly (P < 0.05) increased after addition of EDTA and caffeine as additives when compared with TFYG diluent without any additive (Table). Earlier workers have documented similar beneficial effects of EDTA and caffeine on the semen of cow bulls and buffalo bulls either at refrigerator temperature (Dhami et al., 1993) or at subzero temperature preservation (Singh et al., 1989 and Kumar et al., 2001) or at both (Dhami et al., 1993 and 1995 and Raval and Dhami 2010 in triplebred bulls), where they got improvement in motility and viability of sperms during freezing in presence of semen additive. However, Saxena et al., (1988) could not find beneficial effect of EDTA on storage ability of bovine semen.

**Table. Percentage of individual motility, live sperm, abnormal sperm and acrosomal integrity at different stages of semen cryopreservation.**

<table>
<thead>
<tr>
<th>Semen diluent additives</th>
<th>Individual motility</th>
<th>Live sperm count</th>
<th>Abnormal sperm count</th>
<th>Acrosomal integrity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDS</td>
<td>PES</td>
<td>PTS</td>
<td>PDS</td>
</tr>
<tr>
<td>Control (n=180)</td>
<td>80.59 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.73 ± 0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.83 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.24 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>EDTA (n=180)</td>
<td>84.37 ± 0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.88 ± 0.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62.24 ± 0.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>89.51 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Caffeine (n=180)</td>
<td>83.71 ± 0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77.95 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.17 ± 1.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.13 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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</table>

**Note:** Means with different superscripts within row differ significantly at 5% level.

Raval and Dhami (2010) further reported that functional life span of spermatozoa was enhanced in presence of EDTA by decreased level of glycolytic enzymes and increased oxygen uptake of sperm. EDTA has been a powerful chelating agent and forms stable complex with heavy metals, which chelates with calcium, protects sperm motility and maintains phosphorylation (Raval and Dhami, 2010). EDTA has antibacterial effect on metal chelates in diluted semen.

In the present study motility and viability of spermatozoa were increased with addition of caffeine where immotile sperms due to cold shock could also be mobilized and caffeine also increased the preservation time of bull semen by delaying the loss of sperm motility (Singh et al., 1986). Caffeine has been believed to have stimulatory effect on kinetic activity and respiration of spermatozoa through inhibitions of certain enzymes involved in spermatozoan glycolysis and certain inhibitors of cyclic nucleotide phosphodiesterase responsible for increasing cAMP level. Caffeine also converted inactive form of glycogen phosphorylase into their active form through breaking down of glycogen in to simple sugar, which might be utilized for the longer preservation of bull semen (Singh and Raina, 1999).

In the present investigation, abnormal sperm count was significantly (P < 0.05) decreased and acrosomal integrity of spermatozoa was.
significantly (P < 0.05) higher in EDTA and caffeine groups as compared to control group at the post diluted, post equilibrated and post thawed stages of semen preservation (Table). The results of present investigation were in agreement with the reports of Shannon and Curson (1983) in Jersey bulls, Verma et al., (1999) and Kumar et al., (2001) in Sahiwal bulls and Raval and Dhami (2010) in triplebred bulls; who also reported significantly higher percentage of intact acrosome and decreased percentage of abnormal sperms in post thawed semen with addition of EDTA and/or caffeine in tris diluent as compared to control. However, contrary to present findings Champak et al., (1999) could not find any significant effect on addition of EDTA on acrosomal integrity and abnormal sperm count. Abnormal sperm affect the fertility of male. The admissible limit of abnormal sperm has been less than 10 per cent in normal semen which could be utilized for fertilization and semen with more than 20 per cent abnormal sperm must be discarded (Laying, 1979). A wide variation in abnormal sperm count has been attributed to factors like season, age, testicular diseases and individuality. However, some spermatozoa could be highly motile but not fertile, owing to the acrosomal damage. Intact apical ridge of acrosome has been necessary for fertilizing capacity of spermatozoa and for functional efficiency of the acrosome. Tasseron et al., (1977) reported that acrosome damage might have occurred during dilution, cooling, freezing and thawing processes. Sperm can penetrate the cumulus oophorus cells surrounding the ova by means of the enzyme hyaluronidase and acrocine released from its surface. The acrosomal cap undergoes changes in biochemical composition and ultra-structure during fertilization and acrosomal enzymes play a key role during sperm penetration in zona pellucida. Detachment of acrosome or loss of acrosomal intactness may result in to decreased ATP. Thus it can be concluded that motility, viability and acrosomal integrity of spermatozoa were significantly increased and abnormality was significantly decreased after addition of EDTA and caffeine as additives when compared with control.

REFERENCES


