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**Original Research Article**

# Studies on Certain Physical and Biochemical Attributes of Kankrej Bull Semen

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**A B S T R A C T**

Total 24 semen samples of three healthy Kankrej breeding bulls collected weekly interval for 8 week period by artificial vagina method were evaluated for physical and biochemical attributes from neat and at different stages of cryopreservation viz. post-dilution, post-equilibration and post-thawing. The ejaculate volume averaged to 4.47 ± 0.13 ml, while mass motility were graded to 3.83 ± 0.10 scale and sperm concentration were 1629.04 ± 72.95 million/ml. The individual motility, sperm viability and sperm abnormality were 87.33 ± 0.48, 89.54 ± 0.38 and 3.38 ± 0.22 percent whereas percentage of HOST reactive and acrosomal integrity of spermatozoa were 86.58 ± 0.35and 92.88 ± 0.24, respectively. The level of lipid peroxidation, glutathione reductase, aspartate aminotransferase and alanine aminotransferase in seminal plasma were recorded 49.30 ± 0.81 µmol/ml, 37.11 ± 1.08 U/L, 203.70 ± 12.99 U/L and 85.74 ± 4.21 U/L, respectively. Statistical analysis revealed that the individual motility, viability, HOST reactive, acrosomal intact of spermatozoa and level of aspartate aminotransferase and alanine aminotransferase were significantly higher ($P > 0.05$), whereas percentage of abnormal sperms were significantly ($P > 0.05$) lower in neat semen than all the stages of cryopreservation but their values were decreased as the progress of process except sperm abnormality. The concentration lipid peroxidation was significantly ($P > 0.05$) higher in neat semen seminal plasma than post-diluation and post-equilibration stages and further significantly ($P < 0.05$) increased at post thaw stage, whereas, glutathione reductase level was significantly ($P < 0.05$) lowered in neat seminal plasma than post dilution and post equilibration stages. All the physical attributes except sperm abnormality were greatly reduced as cryopreservation advanced, whereas liberation of enzymes from spermatozoa also increases from post-dilution to post thaw except glutathione reductase.

## Introduction

The Kankrej breed of cattle is a native of Kankrej town in Banaskantha district of North Gujarat. It is very high esteemed, fast, powerful draft cattle and fair enough milk production. Patel and Siddiquee (2013) and Shaikh (2014) proved that the elite Kankrej herd of Livestock research station, Sardarkrushinagar was superior to crossbred cattle in terms of milk production and disease resistance. Due to this property, it needs to conserve the valuable germ-plasm of this indigenous breed due to

indiscriminate crossbreeding with exotic breeds (Dahlin *et al.,* 1998). In keeping view of this fact the National breeding policy of India is focusing on the conservation of indigenous cattle with the help of artificial insemination (AI) to rural areas for the genetic improvement purpose. The artificial insemination has played a major role for the past few decades in the genetic improvement of cattle and buffaloes by increasing selection intensities of males and wide dissemination of their valuable germ plasm after cryopreservation. For this purpose evaluation of seminal characteristics are found to have significant correlation with freezability and fertility of semen (Bhoite *et al.,* 2005). Since physical characteristics of semen alone are not completely satisfactory for semen appraisal in the current practice, hence determinations of some enzymes form seminal plasma are also needed for quality of semen (Mann and Lutwak- Mann, 1981). Hence, the present research was conducted to evaluate the physio-biochemical attributes of Kankrej bull semen from neat and various initial stages of cryopreservation to predict its keeping quality, freezability and fertility.

## Materials and Methods

Three Kankrej bulls aged between 6 to 7 years housed at the Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar and maintained under the uniform conditions were selected for the present study. Each bull was subjected to semen collection weekly interval by artificial vagina. A total of 24 semen ejaculates were obtained in the morning hours (8 to 9 hrs) during the period of 8 weeks. Each ejaculates were divided into two equal amount of aliquot; one utilized for physical and biochemical attributes and second for cryopreservation. All ejaculates were evaluated for physical attributes such as volume, colour and consistency, mass motility, individual motility and sperm concentration (Serpil *et al.,* 2009). Differential staining technique (eosin-nigrosin stain) described by Evans and Maxwell, (1987) was used to determine the number of viable and abnormal spermatozoa. Acrosomal integrity was assessed by means of double stating technique (Kutty *et al.,* 1996). The hypo osmotic swelling test (HOST) was performed as per method described by Revel and Mrode, (1994). The ejaculate containing forward progressive motile spermatozoa more than 80 per cent were used for further process of cryopreservation. The semen was extended with Tris-Fructose Egg yolk citrate glycerol extender to achieve 80 million sperms per ml. Extended semen was loaded into French Mini straw by using automatic filling machine (IS-4, IMV-France) with equilibration at $4^{o}$ C for 4 hours. The programmable bio-freezer (IMV, France) was used for the cryo-freezing and finally semen straws were submerged into liquid nitrogen. The cryopreserved semen was evaluated after 24 hours (post thaw stage).

The seminal plasma was collected from neat semen and cryopreserved semen straws at post dilution, post equilibration and post thawing by centrifuging at 3000 rpm for 10 minutes and evaluated for biochemical properties in relation to certain enzymes. Lipid peroxidation level of seminal plasma was measured by determining the malonaldehyde (MDA) production, using thiobarbituric acid (TBA) as per the method of Placer *et al.,* (1966), whereas, glutathione reductase (GSH) of seminal plasma was measured using the method of Sedlak and Lindsay (1968). Aspartate aminotransferase (U/L) and alanine aminotransferase (U/L) activity were measured by using the Diagnostic Reagent Kits (Reactivos BPL, Barcelona, Espana). The data obtained for

various parameters were analyzed using one way ANOVA followed by the Duncan post hoc test for calculating the Mean ± S.E. for neat and all the stages of cryopreservation to determine significant differences using the methodology described by Snedecor and Cochran (1994).

## Results and Discussion

### Physical attributes

The means ± SE values pertained to various physical characteristics of Kankrej bull semen are depicted in Table-1. Ejaculate volume is probably a breed characteristic, which depends upon the age, season, reproductive health, scrotal size and weight, method and frequency of semen collection and management (Nazir, 1988). The ejaculate volume was found to be 4.47 ± 0.13 ml which is in close agreement with findings reported by Patel and Siddiquee (2013) and Bhavsar (2014) in same breed and by Silva-Mena, (1997), Shelke and Dhami, (2001), Rajoria *et al., (*2011) and Ray and Ghosh, (2013) in other breeds of cattle. However, comparatively higher and lower ejaculate volume than present findings have been reported in Brown Swiss (Cevik *et al.,* 2007) and in Tharparkar bulls (Rajoria *et al.,* 2011), respectively.

The colour and consistency of semen of Kankrej bull were found to be creamy white in 87.50 per cent ejaculates (21/24) whereas, it was milky white in 8.33 per cent (2/24) and watery in 4.17 per cent (1/24) ejaculates, which is in accordance with Bhavsar (2014) in Kankrej bulls; Shelke and Dhami (2001) in Gir bulls and Sharma *et al.,* (2012) in crossbred bulls. Whereas, Patel and Siddiquee (2013) were reported 100 percent ejaculates with creamy white in Kankrej bull. Colour variations in semen might be attributed partly to the low sperm concentration. Few ejaculates of bulls in the present study produced yellowish semen which might be due to the presence of riboflavin in seminal plasma (Hafez, 1980).

Mass motility of sperms has been an important attribute for the acceptance or rejection of the ejaculate for further processing of semen and its use in artificial insemination as it is positively correlated with keeping quality, freezability and fertility of that sample (Bhoite *et al.,* 2005). The finding s of mass motility (3.83 ± 0.10 grade) was in accordance with the findings of Patel and Siddiquee, (2013) and Bhavsar, (2014) in Kankrej bulls, Rana and Dhami, (2004) in Gir bulls, Salah *et al.,* (1992) in HF bulls and Cevik *et al.,* (2007) in Brown Swiss bulls. However, higher scores have been reported in Tharparkar bulls (Rajoria *et al.,* 2011) and HF bulls (Cevik *et al.,* 2007). Whereas, relatively lower mass motility in various other indigenous bulls has been reported (Shelke and Dhami, 2001; Rana and Dhami, 2004; Mandal *et al.,* 2005 and Ray and Ghosh, 2013).

The mean sperm concentration (1629.04 ± 72.95 million/ml) was in agreement with those reported in Kankrej bulls (Patel and Siddiquee, 2013 and Bhavsar, 2014). Comparable values of sperm concentration have been also observed in Sahiwal bulls (Mandal *et al.,* 2005) and HF bulls (Salah *et al.,* 1992). However, higher sperm concentration in Gir bulls (Rana and Dhami, 2004) and relatively lower sperm concentration in indigenous cattle (Silva-Mena, 1997; Shelke and Dhami, 2001; Cevik *et al.,* 2007; Rajoria *et al.,* 2011 and Ray and Ghosh, 2013) has been reported. Such variations are seem to be obvious as the production of spermatozoa is highly specific trait and is influenced greatly by breed, season, age, method of semen collection, testicular size etc.

**Table.1** Physical attributes of neat and at various stages of cryopreservation of Kankrej bull semen (Mean ± SE)

| Stages / Physical attributes | Neat | Post-Dilution Stage (PDS) | Post-Equilibration Stage (PES) | Post-Thaw Stage (PTS) |
|---|---|---|---|---|
| **Ejaculate Volume (ml)** | 4.47 ± 0.13 | --- | --- | --- |
| **Concentration (million/ml)** | 1629.04 ± 72.95 | --- | --- | --- |
| **Mass Motility** | 3.83 ± 0.10 | ---- | ---- | ---- |
| **Individual Motility (%)** | 87.33 ± 0.48 [a] | 76.83 ± 0.40 [b] | 67.67 ± 0.36 [c] | 52.33 ± 0.33[d] |
| **Sperm Viability (%)** | 89.54 ± 0.38 [a] | 79.83 ± 0.38 [b] | 71.29 ± 0.33 [c] | 59.0 ± 0.31[d] |
| **Sperm Abnormality (%)** | 3.38 ± 0.22 [a] | 6.54 ± 0.19 [b] | 8.54 ± 0.18 [c] | 10.21 ± 0.17[d] |
| **HOST Reactive Sperm (%)** | 86.58 ± 0.35 [a] | 75.83 ± 0.50 [b] | 67.79 ± 0.29 [c] | 55.04 ± 0.34[d] |
| **Acrosomal integrity (%)** | 92.88 ±0.24 [a] | 86.88 ± 0.24 [b] | 82.04 ± 0.34 [c] | 73.88 ± 0.50[d] |

Means within row differ significantly at (P < 0.05) level.

**Table.2** Biochemical attributes of neat and at various stages of cryopreservation of Kankrej bull semen (Mean ± SE)

| Stages / Biochemical attributes | Neat | Post-Dilution Stage (PDS) | Post-Equilibration Stage (PES) | Post-Thaw Stage (PTS) |
|---|---|---|---|---|
| **Lipid peroxidation** | 49.30 ± 0.81[a] | 27.57 ± 0.79[b] | 35.57 ± 0.83[c] | 52.77 ± 0.90[d] |
| **Glutathione reductase** | 37.11 ± 1.08[a] | 57.61 ± 0.88[b] | 48.98 ± 0.88[c] | 36.50 ± 1.18[d] |
| **Aspartate aminotransferase** | 203.70 ± 12.99[a] | 82.86 ± 0.97[b] | 116.29 ± 1.41[c] | 157.94 ± 1.49[d] |
| **Alanine aminotransferase** | 85.74 ± 4.21[a] | 18.95 ± 0.77[b] | 31.28 ± 0.86[c] | 48.47 ± 0.92[d] |

Means within row differ -significantly at (P < 0.05) level.

Physical characteristics, such as individual motility, sperm viability and sperm abnormality are the routinely performed tests in semen laboratory for prediction of semen freezeability and fertility rate which helps in acceptance or rejection of semen samples. The mean individual motility, sperm viability and sperm abnormality recorded were 87.33 ± 0.48, 89.54 ± 0.38 and 3.38 ± 0.22 per cent, respectively. The present findings corroborate with the results of Desai (2013); Patel and Siddiquee (2013); Bhavsar (2014) and Shaikh (2014) in Kankrej and Rajoria *et al.*, (2011) in

Tharaparkar bulls. Whilst, a lower but variable mean per cent individual motility, sperm viability and higher sperm abnormality was observed in Gir bulls (Dhami *et al.,* 2003); Sahiwal bulls (Ray and Ghosh 2013); Brahma bulls (Silva-Mena, 1997); Jersey bulls (Dhami *et al.*, 2003) and HF bulls (Cevik *et al.,* 2007). The individual motility and sperm viability were significantly (P > 0.05) reduced progressively in neat semen (87.33 ± 0.48 %; 89.54 ± 0.38 %), post dilution (76.83 ± 0.40 %; 79.83 ± 0.38 %), post equilibration (67.67 ± 0.36 %; 71.29 ± 0.33 %) and post thaw (52.33 ± 0.33 %; 59.00 ± 0.31 %) stages of cryopreservation. However, the concentration of abnormal sperms were greatly increased from 3.38 ± 0.22 % in neat semen to 10.21 ± 0.17 % in post thaw stages at 24 hours of cryopreservation which was near to 3 time increased. This variation in abnormal sperm count could be a one of the reason to drastically decreasing the sperm motility as well as viability besides the other factors. Similarly, different authors also reported the individual motility, sperm viability were significantly (P<0.05) decreased, whereas sperm abnormality was significantly (P<0.05) increased at sub-sequent stages of cryopreservation (Desai, 2013; Patel and Siddiquee, 2013; Bhavsar, 2014 and Rana and Dhami, 2004).

The hypo osmotic swelling ability of spermatozoa has been reported as a sign of membrane integrity and normal functional activity which is not only essential for the maintenance of sperm motility but also for the induction for acrosome reaction and possibly by other event related to fertility (Lodhi *et al.,* 2008). Percentages of HOST reactive sperm was recorded as 86.58 ± 0.35, which was close agreed with findings of Shaikh (2014) in Kankrej bulls. However, the lower values than present findings were obtained by Bhavsar, (2014) in Kankrej bulls. Some researcher recorded the more than 80 percent of HOST reactive sperm in Gir (Rana and Dhami, 2004), in Tharaparkar (Rajoria *et al.,* 2011) and in Sahiwal bulls (Ray and Ghosh, 2013). The percentage of acrosomal intact spermatozoa was found to be 92.88 ± 0.24 which is in close agreement with findings of Patel and Siddiquee (2013) and Bhavsar (2014) in Kankrej and Rajoria *et al.,* (2011) in Tharaparkar and Rana and Dhami, (2004) in Gir bulls. Whilst in Sahiwal bulls lower percent acrosomal integrity has been reported by Ray and Ghosh, (2013). The percentages of HOST reacted and acrosomal integrated spermatozoa (intact) were significantly (P > 0.05) higher in neat semen and subsequently reduced in post dilution, post equilibration and post thaw stages which was in accordance with the findings of Bhavsar (2014) in Kankrej bulls and Dhami *et al.,* (1998) in Jersey bulls. The presence of an intact acrosomal cap and plasma membrane are the important and primary requirement for fertilization process of gametes, so it has been highly related with fertility of particular semen. The positive correlation was seen between the numbers of abnormal sperms was increased with the acrosomal integrity and HOST reacted spermatozoa were decreased.

## Biochemical attributes

The means ± SE values concerned to various biochemical characteristics of Kankrej bull semen are presented in Table-2

LPO in form of Malondialdehyde (MDA) and Glutathione reductase (GSH) level was recorded as 49.30 ± 0.81 µmol/ml and 37.11 ± 1.08 U/L, respectively. The mean MDA values corroborate but, value observed for GSH is higher than the reports of the Shaikh (2014) in Kankrej bulls. The both the parameters are related with the oxidative

stress of spermatozoa or semen. The concentration lipid peroxidation in terma of MDA was significantly ($P > 0.05$) higher in initial seminal plasma than post-diluation and post-equilibration stages and further significantly ($P < 0.05$) increased at post thaw stage, whereas, glutathione reductase level was significantly ($P < 0.05$) lowered in neat seminal plasma than post dilution and post equilibration stages. The present finding were well collaborate with the Bhavsar, (2014) and Shaikh (2014) in Kankrej bulls. Salvador *et al.,* (2006) stated that the oxidative stress is induced by production of free radicals during cryopreservation of semen. Sperm cells have a supplementary content of unsaturated fatty acids in their plasma membranes and they are deficient in cytoplasmic component containing antioxidants. Therefore, sperm cells are highly susceptible to lipid peroxidation (LPO) in presence of ROS, leading to impaired sperm function (Hu *et al.,* 2010). It is well known that GSH plays a critical role in protecting mammalian cells from oxidative damages. Serpil *et al.,* (2009) noted that the elevation of GSH activity was indicative of improved antioxidant capacity but in present study could not found that indicate the spermatozoa under the oxidative stress which can be improved by addition of antioxidant into dilutor.

The overall mean value of AST and ALT activity is 203.70 ± 12.99 U/L and 85.74 ± 4.21 U/L, respectively in the neat semen of Kankrej bulls which was close agreed with the findings by Bhavsar (2014) in Kankrej bulls. AST and ALT level was significantly ($P<0.05$) increased at sub-sequent stages of cryopreservation but it was remained lower than that of the neat seminal plasma which is close agreed with the finding of Patel and Siddiquee (2013), Shaikh (2014) in Kankrej bulls and Rana and Dhami (2004) in Gir bulls. Its levels in seminal plasma are very important for sperm metabolism and sperm function (Brooks, 1990) which provide energy for survival, motility and fertility of spermatozoa and also good indicators of semen quality because they measure sperm membrane stability (Corteel, 1980).

All the physical attributes except sperm abnormality were greatly reduced as cryopreservation advanced, whereas liberation of enzymes from spermatozoa also increases from post-dilution to post thaw except glutathione reductase. Conclusively, Individual motility, sperm viability, HOST reactive sperm and acrosomal integrity per cent were better with less sperm abnormality in Kankrej bull semen than other cattle breeds.

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