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Effect of vitamin-e on sperm mucus penetration distance and sperm morphometry in fresh vs frozen-thawed semen of Kankrej bull

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Abstract
This study applied nine artificially collected ejaculates from three mature Kankrej bulls to study the cryopreservation of semen from mature Kankrej bulls in a tris egg yolk fructose dilutor at varying concentrations (G I: control, G II: 1.5 mM, and G III: 2 mM) of vitamin-E and its impact on spermatozoa penetration within cervical mucus and sperm morphometry. Fresh semen was found to have a sperm mucus penetration distance (SMPD) of 16.78±0.84 mm. Nevertheless, during post-thaw stages of cryopreserved Kankrej bull semen, the overall mean penetration distance of cryopreserved bull spermatozoa was determined to be 19.89±0.63 mm in G I, 26.44±0.82 mm in G II, and 23.00±0.69 mm in G III vitamin-E group, respectively. Compared to other concentrations, the SMPD was considerably ($P < 0.01$) greater in 1.5 mM vit-E. Fresh semen was discovered to include spermatozoa with average head length (HL), head width (HW), and midpiece length (ML) measurements of 8.62 ± 0.04, 4.52 ± 0.03, and 13.98 ± 0.05 µm, respectively. Using different quantities of vitamin-E at the post-thawed stage, the overall mean HL, HW, and ML were 8.56±0.03, 4.44±0.05, and 13.75±0.07 in G I; 8.51±0.03, 4.43±0.04, and 13.72±0.05 µm in G II; and 8.52±0.05, 4.45±0.08, and 13.74±0.06 µm in G III group, respectively.

Keywords: Kankrej bull, cryopreservation, Vitamin-E and sperm mucus penetration distance

1. Introduction
Artificial insemination (AI) has become one of the most widely used and reasonably priced assisted reproductive technologies, with the goal of improving the genetic quality of cattle through the dissemination of superior genes. In rich countries, farmers have begun to utilize it frequently, but it is becoming more and more widespread on cattle in developing nations. AI depends on the generation of high-quality frozen semen. The spermatozoa are subjected to a number of extreme alterations in their chemical and physical environments during cryopreservation, which harms them.

Up to 50% of this harm could occur (Watson, 2000) [1]. The detrimental effects on sperm structure, biochemistry, and functional damage lead to reduced motility, compromised plasma membrane integrity, and lower fertility. The plasma membrane is the main area where cryo-injury to spermatozoa occurs (Krogenaes *et al*., 1994) [2]. Through the activation of lipid peroxidation (LPO) of bio membranes, oxidative stress is recognized to play a significant role in sperm abnormalities (Arabi *et al*., 2001) [3].

Vitamin E, also known as α-tocopherol, is the most significant lipid-soluble and chain-breaking antioxidant that aids in cell defense. By donating the hydrogen from the hydroxyl (-OH) group on the ring structure, it quickly inactivates free radicals. Primarily found in the phospholipid bilayer of cell membranes, it effectively inhibits lipid peroxidation, or the oxidative degradation of polyunsaturated fatty acids (PUFAs) in sperm membranes (Sarangi *et al*., 2017) [4]. Reduced post-thaw motility, viability, membrane integrity, antioxidant status, fertility, and sperm functions have all been linked to excessive reactive oxygen species (ROS) production during cryopreservation (Aitken *et al*. 1998; Bilodeau *et al*. 2000; White, 1993; Zhao and Buhr, 1995) [5, 6, 7, 8].

The *in-vitro* sperm mucus penetration test (SMPT) is a sperm function test used to assess the quality of semen and predict the ability of spermatozoa to fertilize. It measures the ability of sperm in the semen to swim up into a column of cervical mucus or substitute (Anilkumar *et al*., 2001) [9].

2. Materials and Methods

The SMPT and morphometry of fresh and post-thawed spermatozoa cryopreserved in Tris Fructose Egg Yolk dilutor with varied concentration (G I, control; G II, 1.5 mM, and G III, 2.0 mM) of Vitamin-E were studied using a total of 9 ejaculates taken artificially from 3 Kankrej bull.

2.1. Sperm mucus penetration test (SMPT)

Using non-heparinized hematocrit capillary tubes, the Kankrej cow mucus sample with a typical fern pattern and no infection (as determined by the white side test) was used for the SMPT (Matousek *et al*., 1989) [10]. After 45 minutes of incubation, the vanguard spermatozoa in bovine mucus were measured in millimeters.

2.2. Sperm morphometry

Each bull produced three ejaculates, for a total of nine, which were cryopreserved in the neat and post-freeze stages and analyzed for sperm morphometry. As stated by (Gupta and Singh 2018) [11], the slides were prepared for the morphometric examination using the Rose Bengal stain. A phase contrast microscope with a 1000x magnification and a high-resolution digital CCD camera was used to count the 200 spermatozoa that were present in each ejaculate. The data was analyzed using image analysis software (ZEN 2012, Carl Zeiss 3 Microscopy GmbH). Using standard length photographs with defined magnifications and measurement precision of ± 0.1 mm, the system was first calibrated.

3. Statistical analysis

For neat and post-thawed semen, a three factorial CRD (Completely Randomized Design) was employed, followed by the Duncan New Multiple Range Test (DNMRT), to ascertain the differences between various Vit-E concentrations and stages utilizing the approach outlined by (Snedecor and Cochran 1994) [12]. SPSSver.20 (Statistical Packages for Social Sciences) was used for all statistical analyses of the data that were gathered.

3. Results

3.1 SMPT

The neat semen of Kankrej bulls had sperm mucus penetration distance values ranging from 14.83 to 18.73 mm, with an overall mean value of 16.78±0.78 mm. For the K17-98, K17-05, and K18-24 bulls, the mean penetration distance values were 19.00±1.15, 15.67±1.20, and 15.67±1.45. (Table 1).

Table 1: Neat semen sperm mucus penetration test and sperm morphometry (Mean ± S.E.) of Kankrej bulls

Bull	SMPT	Sperm morphometry		
	Penetration Distance (mm/45 min)	Head Length (µm)	Head Width (µm)	Mid-piece length (µm)
K17-98 (n=10)	19.0±1.15	8.55±0.05	4.54±0.04	14.10±0.09
K17-05 (n=10)	15.67±1.20	8.61±0.06	4.51±0.05	13.89±0.07
K18-24 (n=10)	15.67±1.45	8.71±0.09	4.49±0.06	13.95±0.08
Overall (n=30)	16.78±0.84	8.62±0.04	4.52±0.03	13.98±0.05

Table 2 presents the specific results of the penetration distance of vanguard spermatozoa (mm/45 min) of the various Vit-E concentration groups at the post-thaw stage.

Table 2: Effect of various Vit-E concentrations on the mean ± standard deviation of the Kankrej bull spermatozoa in neat and post-thawed semen (mm/45 min)

Neat (n=9)	Post-thaw (n=27)		
	Group I	Group II	Group III
16.78±0.85 [a]	19.89±0.63 [b]	26.44±0.82 [d]	23.00±0.69 [c]

Means bearing different superscripts in a row differ significantly (p<0.01).

Group II had a considerably (p<0.01) greater penetration distance (PD) in comparison to the other groups. There was a significant (p<0.01) variation in PD between Groups I, II, and III, with Group II showing the largest variance. There was a highly significant (p<0.01) difference between Group I, II, III and neat penetration distance and post-thaw penetration distance.

3.2 Sperm morphometry

Table 1. illustrates the mean values of HL, HW, and ML of spermatozoa in fresh semen, indicating a non-significant variation between the bulls. Tables 3 and 4 report the results of sperm morphometry for the various treatment groups at the post-thaw stage.

Table 3: Impact of varying Vitamin E concentrations on sperm morphometry (µm) (Mean ± S.E.) of Kankrej bull spermatozoa during the post-thaw phase

	Group I	Group II	Group III
HL	8.56±0.03	8.51±0.03	8.52±0.05
HW	4.44±0.05	4.43±0.04	4.45±0.08
ML	13.75±0.07	13.72±0.05	13.74±0.06

Table 4: Effect of cryopreservation on Kankrej bull sperm morphometry

	Neat	Post-thaw Overall	P value
HL	8.62±0.04	8.53 ± 0.08	0.111
HW	4.52±0.03	4.44 ± 0.05	0.124
ML**	13.98±0.05	13.74 ± 0.06	0.003

In G I, the mean values of HL, HW, and ML were 8.56±0.03, 4.44±0.05, and 13.75±0.07; in G II, they were 8.51±0.03, 4.43±0.04, and 13.72±0.05; in G III, they were 8.52±0.05, 4.45±0.08, and 13.74±0.06. A non-significant difference in HL, HW, and ML was found between G I, G II, and G III based on statistical analysis. An extremely significant ($P < 0.01$) difference of ML was found between neat and overall mean of post-thawed spermatozoa of all the groups. However, HL and HW did not differ significantly between neat and post-thaw spermatozoa.

4. Discussion

In the present study, post-thawed spermatozoa showed higher SMPD than pristine semen sperm. Thawing-induced cryo-capacitation-like changes during freezing and thawing in the spermatozoa might have affected the distance travelled. The primary characteristics of spermatozoa that influence their capacity to flow through gel or mucus are their post-thaw motility, concentration, percentage of aberrant spermatozoa, and acrosome integrity, according to Galli *et al*., 1991) [13]. The SMPD of spermatozoa that had been cryopreserved at different vit-E concentrations showed a significant increase.

These findings are consistent with (Muzafer *et al*., 2012) [14], who found that 0.3 mg/ml of vitamin E had a significantly stronger effect on spermatozoa penetration distance at the post-thaw stage (50.19± 1.36 percent) compared to the control in crossbred bulls. The changes in sperm motility after thawing, aberrant sperm, and acrosome integrity could be the cause of the variation in sperm penetration distance between groups.

It has been demonstrated that there is a high correlation between the spermatozoa's ability to fertilize and the bull sperm mucus penetration test (SMPT) (Tas *et al*., 2007) [15]. In comparison to the other treatment groups, including the control, G II with 1.5 mM Vit-E demonstrated the maximum penetration distance at the post-thaw stage in the current investigation. The fact that G II travelled a longer distance than the other groups suggests that Vit-E functions as an antioxidant during the cryopreservation of semen at a concentration of 1.5 mM in the dilutor.

It was shown that while HL and HW were unaltered, spermatozoa's ML significantly decreased when semen was cryopreserved. Many writers looked examined the morphometrics of spermatozoa from different species. Higher head length and head width were observed in Jersy bulls (Sundaraman *et al*., 2007), Pandharpuri buffalo bulls (Aggarwal *et al*., 2007), and rams (Bhainsare *et al*., 2018) [16, 17, 18] in comparison to the current study. These results do not support the current findings.

However, after cryopreserved Murrah buffalo bull semen, (Rana *et al*., 2020) [19] found a considerable reduction in sperm mid piece length, which supports the current findings.

5. Conclusion

In to cryopreserve bovine semen, 1.5 mM Vit-E was added to the dilutor. This improved the frozen-thawed semen's quality in terms of sperm mucus penetration. Bovine spermatozoa's mid-piece length shrank as a result of cryopreservation, but the head's length and width remained same.

6. References

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