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Effect of crenellation on different semen characteristics of cattle

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Visual and microscopic evaluation and thereafter selection of bovine semen before and after cryopreservation is very time consuming and needs lot of experience of the observer. Rejection of poor quality semen before cryopreservation can minimize the expenditure in frozen semen production unit. Ejaculates of some breeding bulls after cryopreservation are declared unsuitable for artificial insemination due to poor post thaw motility as fertility rates depend on post thaw motility of frozen semen besides other. Therefore, grading of semen before cryopreservation is an important criterion for higher post thaw motility. Precipitation or crystallization pattern of seminal plasma may be considered as an indicator for higher post-thaw motility. Crystal is composed of sodium chloride and the distinct shape is formed by the proteinaceous compound of seminal plasma (Kihlstrom and Fjellstrom 1969). The distinct shape, termed as crenellation pattern of dried semen was first observed by Verma *et al.* (1982). In some reports, semen quantity as well as quality was judged by observing the crenellation pattern of different ejaculates in breeding bulls (Verma *et al.* 1982, Gupta *et al.* 1990, Mandal *et al.* 2008). Present investigation was planned to study the crenellation patterns of dried semen and its relation with the different quantifiable characteristics and post thaw motility of semen from 5 breeds/ strains of cattle.

The study was conducted at the Frozen Semen Bull Station, Haringhata Farm, Kalyani, West Bengal, India during May to October 2013. Ejaculates (301) from Sahiwal (26), Gir (06), Jersey cross (6), Holstein Friesian cross (3) and Jersey (1) bulls were used for this study. Semen was collected early in the morning by artificial vagina following standard collection procedure. The evaluation was done on the basis of first and second ejaculation. Immediately after collection, a very small drop of semen was taken on a clean grease free slide and kept at room temperature until dried completely for observing the crenellation patterns. The ejaculates were classified according to the crenellation pattern examined by a phase contrast microscope under ×10 and ×100 objectives as per Mandal *et al.* (2008). Subsequently, the semen was subjected to microscopic evaluation for its quality assessment. The initial motility of spermatozoa was observed by a phase contrast microscope under ×20 objective subjectively by a single observer. Sperm concentration was measured by accucell photometer. Post thaw motility (PTM) was examined by thawing 2 straws from each sample at 37ºC in waterbath after 24 h of freezing. PTM was examined at 37ºC subjectively by a single observer using same microscope. Different grades of semen (A1, A2, B1, B2, C1 and C2) as per their crenellation pattern are presented in Figs 1–6. All the samples were classified accordingly.

To study the effect of crenellation pattern on various semen parameters, the data were analyzed by the least square analysis of variance of fitting constant (Harvey 1990). Different sub-groups means were compared by Duncan's multiple range test (DMRT) as per Kramer (1957). Prior to analysis, all the percent values of seminal parameters were transformed to Arcsine values. Logarithmic transformation was used for the concentration of spermatozoa. All the analyses were performed on transformed values. The results were back transformed by taking anti-logarithms of the least-squares means (LSM).

Least squares means along with standard errors of different semen characteristics are given in Table 1. No significant (P>0.05) effect of semen ejaculate numbers on seminal parameters was observed (Table 1). This study revealed that breeds/strains had highly significant (P < 0.01) effect on all the seminal traits and post thaw motility. Semen volume and initial motility were highest in Jersey (6.91±0.10 and 77.3±0.37) and lowest in Jersey cross (3.81±0.04 and 66.2±0.07). The highest and lowest average sperm concentration and PTM was observed in Sahiwal (1486.82±1.10 and 43.7±0.01) and Jersey cross

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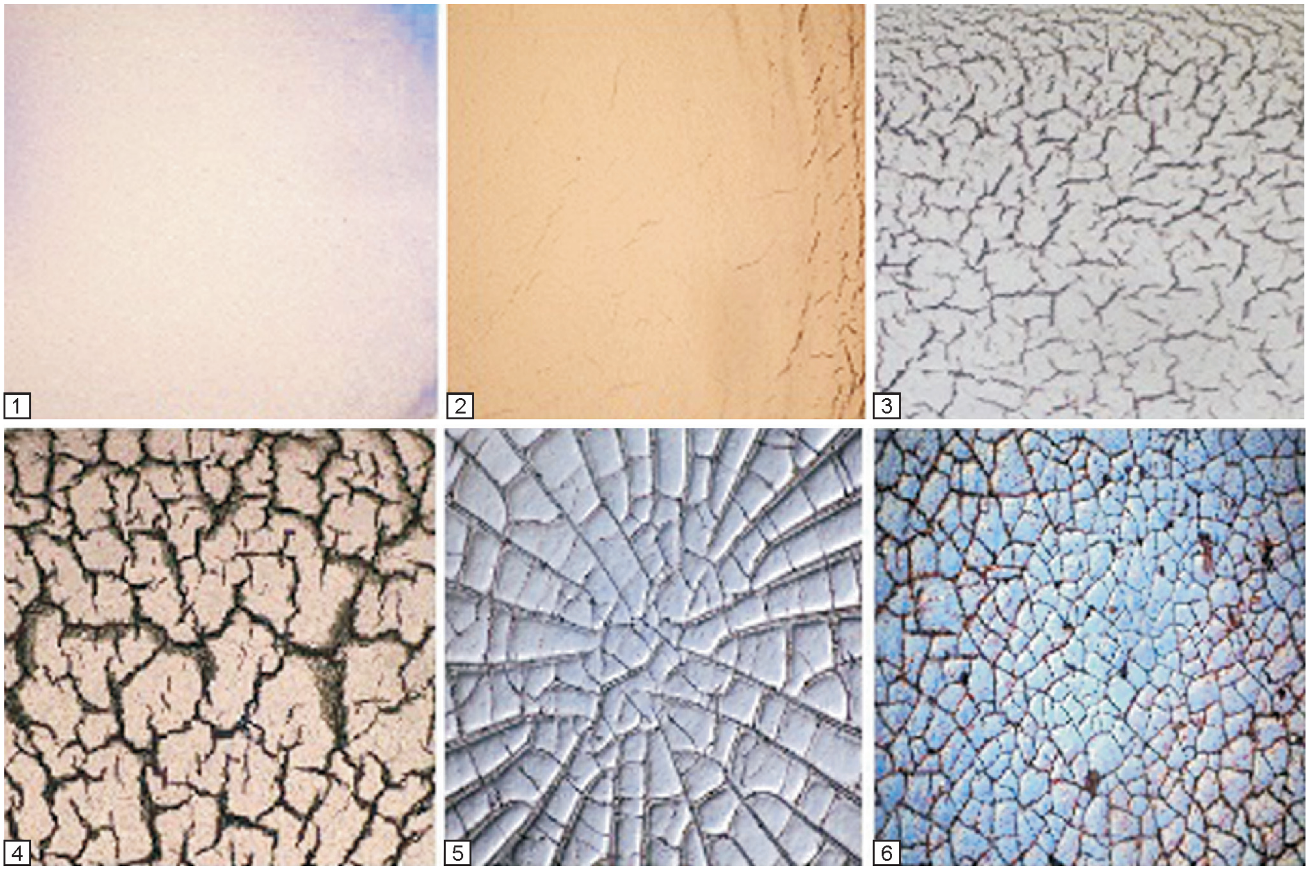
Table 1. Least-square means (± SE) of semen of different breeds of bulls

Parameter	Volume (ml)	Concentration (× 10^6)	Initial motility (%)	PTM (%)
Overall mean	4.70±0.03 (301)	1208.23±1.08 (301)	72.3±0.06 (301)	42.2±0.02 (276)
Ejaculate no.	NS	NS	NS	NS
First	4.84±0.02 (287)	1237.74±1.23 (287)	71.5±0.03 (287)	41.3±0.01 (262)
Second	4.55±0.05 (14)	1179.43±1.13 (14)	73.1±0.15 (14)	43.1±0.05 (14)
Breeds/strains of cattle	**	**	**	**
Sahiwal	4.86±0.03[b] (202)	1486.82±1.10[a] (202)	73.7±0.04[c] (202)	43.7±0.01[a] (187)
Gir	4.40±0.04[c] (36)	1147.70±1.10[c] (36)	74.9±0.08[b] (36)	43.2±0.03[b] (33)
Jersey Cross	3.81±0.04[d] (45)	989.90±1.13[e] (45)	66.2±0.07[e] (45)	38.0±0.03[d] (40)
HF Cross	3.52±0.10[e] (13)	1055.66±1.13[d] (13)	69.9±0.17[d] (13)	43.8±0.06[a] (11)
Jersey	6.91±0.10[a] (5)	1443.92±1.14[b] (5)	77.3±0.37 [a] (5)	42.4±0.13[c] (5)
Months of collection	NS	*	NS	*
May	5.34±0.10 (13)	1481.12±1.14[a] (13)	75.5±0.18 (13)	46.5±0.07[a] (11)
June	4.65±0.03 (72)	1161.47±1.08[d] (72)	74.1±0.06 (72)	43.8±0.02[b] (67)
July	4.75±0.03 (122)	1173.88±1.08[c] (122)	71.2±0.06 (122)	40.4±0.02[d] (105)
Aug	4.38±0.05 (29)	1320.60±1.13[b] (29)	70±0.11 (29)	42.7±0.04[c] (28)
Sept	4.61±0.06 (16)	1145.25±1.13[e] (16)	71.2±0.15 (16)	40.5±0.05[d] (16)
Oct	4.44±0.04 (49)	1018.61±1.13[f] (49)	71.5±0.09 (49)	39.4±0.03[e] (49)
Crenellation patterns	NS	**	**	**
A1	4.14±0.05 (16)	1508.73±1.13[c] (16)	82.4±0.15[a] (16)	55.1±0.05[a] (15)
A2	4.65±0.04 (53)	1621.65±1.10[a] (53)	81.1±0.09[b] (53)	52.6±0.03[b] (50)
B1	4.95±0.04 (100)	1532.36±1.10[b] (100)	79.1±0.17[c] (100)	50.4±0.03[c] (95)
B2	4.78±0.04 (89)	1299.43±1.11[d] (89)	72.0±0.07[d] (89)	41.0±0.03[d] (75)
C1	4.78±0.05 (30)	798.15±1.11[e] (30)	59.9±0.11[e] (30)	29.0±0.04[e] (28)
C2	4.88±0.06 (13)	800.90±1.00 [e] (13)	55.7±0.17[f] (13)	26.4±0.06[f] (13)

Means with different superscript within each column differ significantly (P< 0.05), figures in parenthesis are number of observations, * (P< 0.05), ** (P< 0.01), NS, nonsignificant.

(989.90±1.13 and 38.0±0.03) respectively. Significant variations in semen characteristics among different breeds of cattle were also reported earlier (Bhoite *et al.* 2008, Gupta *et al.* 1990). The pure breeds like Jersey, Sahiwal and Gir generally characterized the better semen quality parameters compared to cross bred bulls in this study. Similar findings were reported by Bhakat *et al.* (2009). Significant (P<0.05) variations in sperm concentration and PTM of bulls were observed among the months of semen collection in this study, however the month of collection had non significant effect on volume of semen and initial motility. The highest sperm concentration (1481.12±1.14) and PTM (46.5±0.07) was observed in May (summer) whereas the lowest sperm concentration (1018.61±1.13) and PTM (39.4±0.03) occurred in the month of October (autumn). The highest percentage of initial motility was in May and June (summer). The lowest value of this feature coincided with the period from July to October (monsoon and autumn). Seasonal variations were also reported by other workers in semen quality (Bhakat *et al.* 2009, Snoj *et al.* 2013) and PTM (Orgal *et al.* 2012) in different breeds of cattle. High rainfall, humidity and insufficient green fodder from



Figs. 1-6. Crenellation patterns of dried bovine semen. **1**, Grade A1; **2**, Grade A2; **3**, Grade B1; **4**, Grade B2; **5**, Grade C1; **6**, Grade C2.

monsoon to early autumn (July to October) may be responsible for lower sperm concentration, initial motility and PTM in the present study. Bhakat *et al.* (2009) also reported that semen quality is affected by different climatic factors like temperature, humidity, rainfall and photo-periods.

The crenellation patterns of semen had highly significant ($P < 0.01$) effect on seminal characteristics and PTM except semen volume (Table 1). Significant variations of sperm concentration, initial motility and PTM were existed among the different grades of semen. The highest and lowest average sperm concentration was found in grade A-2 (1621.65±1.10) and C1 (798.15±1.11) respectively. Sometimes C1 and C2 grade semen was observed as oligospermic with watery to milky white consistency. Similar observations regarding sperm concentration were also reported by Verma *et al.* (1982) and Mandal *et al.* (2008) in crossbred bulls. A1 (82.4±0.15), A2 (81.1±0.09), B1 (79.1±0.17) and B2 (72.0±0.07) grade had > 70% average initial sperm motility. On the contrary, C1 (59.9±0.11) and C2 grade (55.7±0.17) had < 60% average initial motility. Similar findings of lower initial motility of sperm in grade C1 and C2 were also reported by Verma *et al.* (1982) and Mandal *et al.* (2008). The study also depicted that A1, A2 and B1 grade semen had > 50% average PTM, whereas, less than 50% the average PTM was recorded in B2, C1 and C2 grade (Table 1). A minimum of 70% initial motility and 50% PTM is desirable for production and use of frozen semen as per recommendations of Minimum standard protocol (GOI, MSP, 2012). Present study revealed differences in crenellation patterns of semen in different breeds/strains of cattle. However, reason for such difference is not known. It is anticipated that differences in concentration of protein-nitrogen compounds, Cl^- and Na^+ ion may be responsible for the formation of such crenellation patterns as indicated by Kihlstrom and Fjellstrom (1969). Lower initial motility and sperm concentration of C1 and C2 grade semen in this study may be responsible for poor PTM of frozen semen. As the crenellation pattern had significant effect on different seminal traits and PTM, the crenellation patterns can be effectively utilized for assessment of semen quality before accepting it for cryopreservation.

SUMMARY

The present study was conducted to study the crenellation patterns of semen and their relation with different seminal characteristics and post-thaw motility. Dried semen samples were classified into 6 grades (A1, A2, B1, B2, C1 and C2) based on the crenellation patterns. The study showed that breeds/strains had highly significant ($P< 0.01$) effect on different seminal traits. Significant ($P< 0.05$) effect of month of collection on sperm concentration and post- thaw motility was also observed. Crenellation patterns had highly

significant (P< 0.01) effect on sperm concentration, initial motility and post-thaw motility. Sperm concentration, Initial motility and post-thaw motility was superior in A1, A2 and B1 grade and inferior in C1 and C2. B2 grade was of intermediate quality semen. A1, A2 and B1 grade ejaculates gave best post-thaw motility after cryopreservation. Poor post-thaw motility was found in C1, C2 and B2 grade ejaculates. Thus the crenellation can be effectively used as an indicator for simple and quick evaluation of semen quality in any semen laboratory as well as, at field level.

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