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Evaluation of the sperm migration capacity of crossbred dairy cattle bull semen vis-à-vis field fertility trials

HIMANSHU BHATIA[1], PRAHLAD SINGH[2], and S S SIDHU[3]

Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab 141 004 India

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ABSTRACT

Crossbred dairy cattle bulls (6) were subjected to semen evaluation study twice a week for sperm migration capacity in bovine cervical mucus (BCM) and poly-acrylamide gel (PAG). Cervical mucus from respective cows subsequently inseminated with same semen, was tested for sperm penetration test (SPT). PAG was prepared according to standard procedures and stored at 4°C till further use. Different grades of PAG (2% and 3%) were examined for transparency, spinbarkeit, pH and visible gelification before using for sperm migration assay. Field fertility trials based on pregnancy outcome by assigning 10 cows/bull were conducted simultaneously to BCM penetration test (BCMPT) and PAG-SPT. 2% PAG was better in assessing sperm penetration distance than 3% PAG, however, the values differed nonsignificantly. Sperm migration in BCM was better than sperm migration in PAG, and differences were significant for semen of respective bulls. The pregnancy outcome from the bull (FC-1207) having maximum sperm penetration distance (SPD) in BCM (fresh semen, 38.63 ± 0.26 mm/20 min; extended semen, 37.39 ± 0.19 mm/20min; frozen semen, 36.85 ± 0.21 mm/20min), 2% PAG (fresh, 37.44 ± 0.54 mm/20min; extended, 36.60 ± 0.34 mm/20 min; frozen, 35.89 ± 0.44 mm/20min) were around 58.53%, whereas, bull showing lower values of SPD in BCM and 2% PAG had lower pregnancy outcome. Six bulls under study indicated a lot of individual variation for SPD and pregnancy outcome, highlighting lower pregnancy outcome for bulls showing less SPD of sperm from fresh and frozen semen. Positive correlations were exhibited between different penetration tests and pregnancy outcome as well as between BCMPT and PAG-SPT. PAG can thus substitute for bovine cervical mucus and for assessing the sperm migration ability of various bulls used for breeding programme beforehand. Positive correlations between these tests and pregnancy outcome based on field fertility trials indicates importance of these tests for evaluating the fertility of bulls particularly with PAG-SPT before subjecting them to field evaluation.

Key words: Cattle, Cervical mucus penetration test, Fertility trials, Polyacrylamide gel, Semen

Conventional semen evaluation approaches are not sufficient for prediction of fertilizing potential of a bull. Various physical methods proposed to evaluate semen quality of a bull, viz. volume, sperm count, viability, pH and morphological examination of semen ejaculate have a low prediction value for fertility assessment.

Bovine cervical mucus penetration test (BCMPT) and polyacrylamide gel-sperm penetration test (PAG-SPT) are effective methods to predict the sperm migration capacity and hence the fertility of crossbred bulls. BCMPT directly relates to fertility (Murase *et al.* 1990) by predicting viability and forward progressive movement of the sperm, thus confirming the spermatozoa arrival at the site of fertilization. Sperm migration in female genital tract depends on sperm motility, genital contraction and biophysical characteristics of cervical mucus (Panchal 1988, Overstreet and Katz 1997). Chemical and physical changes in cervical mucus affect the sperm transport from site of ejaculation to site of fertilization (Moghissi 1972, Zavos and Correa 1980). An effective replacement for cervical mucus is polyacrylamide gel (PAG) which can be stored qualitatively for a longer time, overcomes the limitation of individual variation and other factors.

The present study was planned with the objective to evaluate the sperm migration capacity by bovine cervical mucus penetration test (BCMPT) and polyacrylamide gel sperm penetration test (PAG-SPT), and comparative assessment based on conception rates achieved under field conditions through artificial insemination.

MATERIALS AND METHODS

Study was carried out on six cross-bred (Holstein Friesian × Sahiwal) cattle bulls of 2.5 to 4.0 years of age managed under similar conditions of nutrition, feeding and

Present address: [1]Veterinary Officer (vhbeetan@gmail.com), Beetan, Haroli, Una, Himachal Pradesh.

[2]Physiologist (prahlad.gadvasu@gmail.com), Department of Animal Genetics and Breeding; [3]Professor (drsidhu@yahoo.com), Department of Veterinary Clinical Services Complex.

management at GADVASU Dairy farm, Ludhiana, India.

Semen samples were collected twice weekly from the bulls by artificial vagina technique; 8 ejaculates/bull were extended with egg yolk citrate agar and evaluated for BCMPT and PAG-SPT.

Bovine cervical mucus penetration test: Cervical mucus was collected from 20 crossbred cows subsequently inseminated by the same bull semen in the standing estrus under hygienic conditions for cervical mucus penetration test (Murase *et al*. 1990). Mucus fern pattern showing typical tertiary/quaternary pattern was used for BCMPT.

Semen-mucus preparation was incubated for 20 min at 38°C on the heated plate to allow spermatozoa to penetrate mucus in the capillary tube. At the end of incubation period the capillary tube was immersed into a 60°C water bath for 5 min to stop sperm motility and stored at 4°C in the refrigerator till further observations.

The distance from the open end was defined as the penetration distance. Each semen sample was tested in duplicate using an aliquot of semen from the semen storage vial. The results were expressed on the average of these two distance measurements in mm.

Poly acrylamide gel – sperm penetration test (PAG-SPT): Sperm penetration distance using synthetic media was conducted as per the method described by Kaushal *et al.* (2005).

Field fertility trials: Frozen semen of crossbred bulls under investigation was used in field to inseminate healthy, normal cycling cross - bred cows (10 cows/bull). Cows were inseminated at mid-estrus and pregnancy was checked after 60 days of each rectal findings. Pregnancy rates (conception/insemination) were used as an indicator of fertility status for the respective bulls.

Statistical method: The data generated from the present study was subjected to standard statistical methods. Briefly RBD with homogenization of data and ANOVA to evaluate the significance of outcomes were applied.

RESULTS AND DISCUSSION

Sperm migration in bovine cervical mucus: Maximum sperm migration was recorded for the spermatozoa from fresh semen followed by extended and then frozen-thawed semen (Table 1). Sperm migration using fresh, extended and thawed semen in bovine cervical mucus indicated significant differences (P<0.05). Migration capacity of sperm within the bulls also varied significantly (P<0.05).

Activity of sperm migration using bovine cervical mucus (BCM) as the media for observing the SPD (mm/20 min) in the present study were on higher side in most of the bulls as compared to the earlier studies.

Sperm migration in polyacrylamide gel: The sperm migration capacity is given in Table 2. The values of sperm migration were higher in PAG 2% as compared to PAG 3%. Within the bulls individual variation regarding sperm migration in PAG indicated significant (P<0.05) differences. Migration rates in 2 concentrations of PAG differed nonsignificantly for all the bulls (Table 3). Bulls showing lower sperm migration in PAG 2% also had lower values in PAG 3%. A nonsignificant difference was observed for migration rates in PAG 2 and 3%. However, between bulls the differences in migration rates were significant (P<0.05) in the 2 concentrations of PAG (Table 4).

Table 1. Bovine cervical mucus penetration (mm/20min) test (BCMPT) using fresh, extended and frozen-thawed semen of cross bred dairy cattle bulls

Bull no.	BCMPT (mm/20 min)			
	Fresh semen	Extended semen	Thawed semen	CD
FC-1238	36.35 ± 0.17	35.83 ± 0.17	34.70 ± 0.16	0.41
FC-1256	33.63 ± 0.23	32.93± 0.25	32.13 ± 0.26	0.59
FC-1209	26.33 ± 0.16	25.84 ± 0.18	24.83 ± 0.20	0.78
FC-1207	38.63 ± 0.26	37.39 ± 0.19	36.85 ± 0.21	1.01
FC-1218	21.06 ± 0.15	20.45 ± 0.13	19.99 ± 0.11	0.46
FC-1230	15.80 ± 0.19	15.30 ± 0.25	14.86 ± 0.25	0.68
CD	1.20	1.14	1.44	

Data presented as mean ± SEM; n, 8; critical difference (CD), t-value significant at (P<0.05).

Table 2. Polyacrylamide gel sperm penetration test (PAG-SPT) (mm/20min) using fresh semen of cross bred dairy cattle bulls

Bull No.	SPD (mm/20 min)		t-value
	PAG 2%	PAG 3%	
FC-1238	35.38 ± 0.46	34.89 ± 0.47	0.74
FC-1256	31.49 ± 0.42	31.03 ± 0.43	0.77
FC-1209	23.29 ± 0.45	22.76 ± 0.46	0.82
FC-1207	37.44 ± 0.54	36.84 ± 0.53	0.79
FC-1218	19.75 ± 0.43	19.16 ± 0.43	0.96
FC-1230	14.99 ± 0.44	14.07 ± 0.38	1.58
CD	1.21	1.22	

Data presented as mean ± SEM; n, 8; critical difference (CD), t-value significant at (P<0.05).

Various crossbred bulls in the present study showed wide variation in sperm penetration distance in PAG, whereas, the respective results for the 2 concentrations of PAG used did not show significant differences for these bulls. PAG 2% being of low consistency as compared to 3% had slightly more sperm migration in all the bulls. The values obtained for fresh, extended and frozen thawed semen in terms of sperm penetration distance indicated similar pattern in the 2 concentrations of PAG. The present findings were in consistent with those of Kaushal *et al.* (2005), Goldstein *et al.* (1982), Eggert-Kruse *et al.* (1993).

Sperm migration capacity in cervical mucus versus synthetic media (PAG): Sperm migration distance was higher in BCM as compared to PAG (Table 5). Significant (P<0.05)

Table 3. PAG Sperm penetration (mm/20min) using extended semen of cross bred dairy cattle bulls

Bull No.	PAG 2%	PAG 3%	t-value
FC-1238	34.56 ± 0.49	34.18 ± 0.49	0.56
FC-1256	30.68 ± 0.46	29.75 ± 0.75	1.05
FC-1209	22.01 ± 0.48	21.65 ± 0.43	0.57
FC-1207	36.60 ± 0.34	36.01 ± 0.50	0.88
FC-1218	18.64 ± 0.38	18.30 ± 0.41	0.60
FC-1230	13.74 ± 0.27	13.36 ± 0.24	1.05
C D	1.18	1.38	

Data presented as Mean ± SEM; n, 8; critical difference (CD) and t-value significant at P<0.05.

Table 4. PAG Sperm penetration (mm/20min) using frozen thawed semen of cross bred dairy cattle bulls

Bull No.	PAG 2%	PAG 3%	t-value
FC-1238	33.71 ± 0.41	31.29 ± 1.83	1.29
FC-1256	29.31± 0.76	28.88 ± 0.76	0.41
FC-1209	21.26 ± 0.45	20.68 ± 0.55	0.83
FC-1207	35.89 ± 0.44	35.24 ± 0.51	0.97
FC-1218	17.71 ± 0.39	17.15 ± 0.38	1.01
FC-1230	12.98 ± 0.29	12.43± 0.26	1.41
CD	1.36	2.52	

Data presented as Mean ± SEM; n, 8; critical difference (CD) and t-value significant at P<0.05.

Table 5. Sperm penetration (mm/20min) tests using PAG and BCMT for evaluating the fresh semen of cross bred dairy cattle bulls

	PAG 2%	BCMPT
FC-1238	35.38 ± 0.46	36.35 ± 0.17
FC-1256	31.49 ± 0.42	33.63 ± 0.50
FC-1209	23.29 ± 0.45	26.33 ± 0.46
FC-1207	37.44 ± 0.54	38.63 ± 0.26
FC-1218	19.75 ± 0.43	21.06 ± 0.36
FC-1230	14.99 ± 0.44	15.80 ± 0.19
CD	3.57	2.91

Data presented as Mean ± SEM; n, 8; critical difference (CD) and t-value significant at P<0.05.

Table 6. Sperm penetration (mm/20min) tests using PAG and BCM for evaluating the extended semen of crossbred dairy cattle bulls

Bull No.	PAG 2%	BCMPT
FC-1238	34.56 ± 0.49	35.83 ± 0.17
FC-1256	30.68 ± 0.46	32.93± 0.25
FC-1209	22.01 ± 0.48	25.84 ± 0.18
FC-1207	36.60 ± 0.34	37.39 ± 0.19
FC-1218	18.64 ± 0.38	20.45 ± 0.13
FC-1230	13.74 ± 0.27	15.30 ± 0.25
CD	3.18	2.89

Data presented as Mean ± SEM; n, 8; critical difference (CD) and t-value significant at P<0.05.

Table 7. Pregnancy outcome (%) in light of various tests applied to evaluate frozen semen of crossbred dairy cattle bull

Bull no.	PAG 2% (mm/20 min)	BCMPT (mm/20 min)	Pregnancy rate (%)
Fc-1238	33.71 ± 0.41	34.70 ± 0.16	54.50
Fc-1256	29.31± 0.76	32.13± 0.26	50.00
Fc-1209	21.26 ± 0.45	24.83± 0.20	46.66
Fc-1207	35.88 ± 0.44	36.85± 0.21	58.53
Fc-1218	17.71 ± 0.39	19.99 ± 0.11	30.00
Fc-1230	12.98 ± 0.29	14.86± 0.25	25.00
CD	3.68	3.09	

Data presented as Mean ± SEM; n, 8; critical difference (CD) and t-value significant at P<0.05.

Table 8. Correlation coefficients of various evaluation tests with pregnancy outcome

Tests	Concentrations	Semen	Correlation coefficients (r)
PAG	2%	Fresh	0.967
	3%	Fresh	0.966
	2%	Extended	0.966
	3%	Extended	0.962
	2%	Frozen	0.966
	3%	Frozen	0.946
BCMPT		Fresh	0.969
		Extended	0.963
		Frozen	0.963

Critical value of r at 5%, 0.284.

differences were observed in sperm migration values for within the bulls for PAG-SPT and BCMPT. Similarly significant differences (P<0.05) were observed between the values for PAG-SPT and BCMPT. The present findings are similar to those of Kauhsal *et al.* (2004), Kaushal *et al. (*2005), Lorton *et al. (*1981), Goldstein *et al. (*1982), Eggert-Kruse *et al. (*1993) and Tang *et al.* (1999).

Sperm penetration was higher in BCM than in PAG (Table 6). Significant differences (P<0.05) were observed within the sperm penetration values for bulls subjected to PAG-SPT and BCMPT. The differences in sperm penetration distance values after PAG-SPT and BCMPT were also significant (P<0.05).

Sperm migration after BCMPT were higher as compared to PAG-SPT (Table 7). Significant differences (P<0.05) were observed between the PAG-SPT and BCMPT for sperm migration distance.

Pregnancy outcome versus various tests: In the present investigation an effort was made to study the field trials regarding pregnancy outcome from the semen of the bull subjected to various tests (Table 7). The bull having higher sperm migration in PAG 2% (35.88 ± 0.44 mm/20 min) and bovine cervical mucus (36.85 ± 0.21 mm/20 min) had maximum pregnancy outcome in terms of % pregnancy.

Relationship of different tests with pregnancy outcome: Highest positive correlation was observed between the pregnancy outcome and sperm migration in various concentrations (Table 8) of PAG (0.94 to 0.97) as well as in BCM (0.97) for all types of semen used under the present study. The high correlation indicates the applicability and usefulness of these sperm migration tests for evaluation for semen of various bulls.

The high correlation observed in the present investigation between the sperm migration capacity and pregnancy outcome are in close consistency to the findings of Predojevic and Miljkovic (1984), Murase *et al.* (1990) and Suttiyotin *et al.* (1991) who observed positive correlation between bull fertility and SPD for frozen semen. On the other hand Kummerfield *et al.* (1981), Matousek *et al.* (1989), Galli *et al.* (1991) and Das *et al.* (1999) who recorded a non-significant correlation between sperm migration and conception rate. The variation in findings of different workers may be attributed to small sample size and difference in materials and methods.

The correlations between PAG and BCMPT recorded (0.98) in present investigation, indicate that sperm has equal ability to penetrate PAG as well as cervical mucus. Hence PAG can be used as an alternate for sperm migration capacity tests as observed for cervical mucus irrespective of the cow thus overcoming the limitations of individual variation and stage of estrus at which the cervical mucus is obtained for BCMPT.

From the present study it was concluded that the sperm migration capacity based on BCMPT and PAG-SPT is valuable in predicting fertility status of a bull. The pregnancy outcome was better for the bull having better sperm migration in BCM and PAG. The high positive correlation between the penetration tests applied and fertility status of bulls specifies the role of these tests in establishing predictive parameters.

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