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Evaluation of polyacrylamide gel as a substitute for bovine estrual mucus for sperm migration studies in buffaloes

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ABSTRACT

Sperm penetration test was conducted by using different grades of polyacrylamide gel (PAG) i.e. 1%, 2%, 3% and 4% as penetration media. The effect of duration and temperature of storage of PAG on sperm penetration distance (SPD) was recorded. The 1% and 4% PAG were found to be not suitable for sperm penetration studies, 1% being very thin and 4% being too thick. However, 2% and 3% PAG showed results comparable to fresh oestrual mucus. The mean SPD values in 2% and 3% PAG were 20.5±0.87 and 18.80±0.60 mm/20 min, respectively. The duration and temperature of storage showed no significant effect on SPD values after 60 days in 2% and 3% PAG. It was concluded that 2% or 3% PAG could be used as an alternative medium to evaluate the *semen quality of buffalo bulls.*

Key words : Polyacrylamide gel, estrual mucus, sperm migration

Cervical mucus from cows and buffaloes have been used as a test medium for sperm penetration *in vitro* and for evaluation of bull fertility (Dev *et al.*, 1996). To conduct sperm migration study, cervical mucus has to be collected under standardized conditions and the quality of mucus must be carefully controlled to obtain reproducible results and to evaluate the male factor as main variable (Eggert kruse *et al.*, 1989). Also in adrological practice fresh cervical mucus for sperm mucus penetration test is not always available. To overcome the problem an altenative media is needed with sperm migration characteristic similar to those of estrual mucus which could be obtained or prepared freshly with uniform stable characteristics and can also be stored for longer periods without deterioration in quality. Lorton *et al.* (1981) proposed the use of polyacrylamide gel (PAG) as a substitute for cervical mucus in human migration studies. Therefore present study was undertaken to seek and evaluate synthetic media (PAG) for qualitative studies on buffalo bull spermatozoa.

MATERIALS AND METHODS

The synthetic migration media i.e. polyacrylamide gel was prepared as per method of Lorton *et al.* (1981). Four different grades of PAG i.e. 1%, 2%, 3% and 4% were selected for studies. Each grade of PAG was divided into 7 groups. Group-I included samples which were subjected to sperm penetration test on day of preparation. Group-II and Group-III included gel samples which were subjected to sperm penetration test after 4 days of storage at temperature of +4°C and -20°C respectively. Group IV and V included same gel samples but subjected to sperm penetration test after 8 days of storage at +4°C and -20°C respectively. Group VI and VII included same gel samples but stored for 60 days at +4°C and -20° C respectively. Three different sets of each conc. of PAG were prepared and used for sperm migration studies. Frozen semen straws having initial motility 80% and post thaw motility > 40% from the bull of known fertility were used to conduct all the sperm penetration studies. The sperm migration studies in fresh as well as stored mucus samples were conducted as per method described by Kremer (1965) with migration media having various concentration of PAG instead of estrual mucus. The distance travelled by foremost spermatozoa in PAG was measured with the scale fixed on microscope. SPD values were recorded in mm/20 min.

RESULTS AND DISCUSSION

The SPD values for different gradesof PAG stored for different periods of time at different temperatures are given in Table 1. The studies could not be conducted in 4% PAG due to excessive gelification which made it difficult to

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Table 1. Mean sperm penetration distance (SPD) values of PAG samples (1%, 2% and 3%) stored at different temperatures for different periods of time and their correlation with duration of storage

Grade of PAG	Temp of storage	Mean sperm penetration in different grades of PAG when stored over different periods of time (mm/20 min.) Day `0'	Day `4'	Day `8'	Day `60'	Overall mean	Correlation coefficient between SPD values and duration of storage w.r.t. different grades of PAG
1%	+ 4° C	–	14.6±0.33 (3)	14.6±1.20 (3)	15.0±1.15 (3)	14.3±0.44 (3)	
	–20°C	–	15.3±1.16 (3)	14.6±1.20 (3)	14.0±1.15 (3)	14.7±0.62 (3)	–0.33*
	Overall Mean	13.5±1.32 (3)	15.0±0.56 (6)	14.7±0.76 (6)	13.8±0.60 (6)	–	
2%	+ 4°C	–	20.3±1.30 (3)	22.2±2.92 (3)	20.0±1.52 (3)	20.8±1.08 (6)	
	–20°C	–	21.0±1.15 (3)	22.3±2.33 (3)	22.0±1.00 (3)	21.8±0.83 (6)	–0.01*
	Overall Mean	20.5±0.87 (3)	20.7±0.79 (6)	22.3±1.67 (6)	21.0±0.13 (6)	–	
3%	+ 4°C	–	20.0±1.32 (3)	19.0±0.29 (3)	19.8±0.60 (3)	19.6±0.46 (3)	
	–20°C	–	20.3±1.36 (3)	19.0±0.58 (3)	18.7±0.89 (3)	19.3±0.56 (3)	–0.01*
	Overall Mean	18.80±0.60 (3)	20.2±0.85 (6)	19.0±0.29 (6)	19.3±0.54 (6)	–	

* Non-significant at 5% level

be sucked in capillary tube. The mean SPD values in freshly prepared 1% PAG and after 60 days of storage varied non-significantly. The spermatozoa penetrated in 1% PAG in good number but parallel fashion of migration was not evident. The SPD values were also less as compared to 2% and 3% PAG. This might be due to the lower conc. of acrylamide which resulted in improper cross links among the various molecules of gel and hence reduced penetration. A negative non-significant correlation (-0.33) was observed between SPD values and duration of storage (Table 1). There was non-significant variation in 1% PAG samples stored at +4°C and -20°C (Table 1).

In 2% PAG there was non-significant variation of mean SPD value in fresh and samples stored for 60 days. The spermatozoa penetrated in good number with parallel orientation. Lorton *et al.* (1981) reported slightly higher SPD values with human spermatozoa. Temperatures of storage showed no significant effect on SPD values and period of storage. Similar type of findings were reported by Lorton *et al.* (1981) after 4 and 8 days of storage.

The overall average SPD values for freshly prepared 3% PAG and after 60 days of storage were found to be 18.8±0.60 and 19.3±0.54 mm/20 min. Mean SPD values were found to 19.6±0.40 and 19.3±0.56 mm/20 min. for the samples stored at +4°C and -20°C, respectively, the difference being non-significant. Spermatozoa in 3% PAG penetrated in similar fashion as in 2% but the SPD values were slightly lower than in 2% PAG. The decrease in SPD values might be due to increased conc. of Acrylamide in 3% PAG which resulted in increased gelification which in turn resist the penetration of spermatozoa. The spermatozoa penetrated the 3% PAG in parallel fashion with fair conc. of spermatozoa in gel. A very low negative correlation (-0.01) was observed between the SPD values and duration of storage (Table 1).

Both 2% and 3% PAG served as better substitute for cervical mucus with additional advantage of easy preparation and storage without deterioration in quality for at least 2 months. There is more clear visiblity of spermatozoa in PAG as compared to mucus where visibility of foremost spermatozoa is difficult. The 2% media proved to be more useful due to its thinner consistency. Similar results were reported by Lorton *et al.* (1981). Goldstein *et al.* (1982) also concluded that 1.8% PAG was more useful for conducting *in vitro* studies on sperm penetration.

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