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Short Communication

A comparative study on sperm penetration in estrual mucus and polyacrylamide gel in relation to seminal characteristics

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ABSTRACT

In vitro sperm mucus penetration study was done in freshly collected estrual mucus of buffaloes and in synthetic migration media i.e. polyacrylamide gel (2%). Sperm mucus penetration distance was measured as an indicator of fertility. Sperm mucus penetration distance values have +ve correlation with live and normal sperm count. In polyacrylamide there was +ve correlation between sperm penetration distance value and normal sperm count. Polyacrylamide gel can be used successfully as a better substitute penetration media for sperm penetration for assessing the fertility of the semen.

Key words: Sperm, estrual, mucus, polyacrylamide, penetration , fertility

The fertilizing ability of the spermatozoa can be ascertained by *in vitro* fertility tests. The routinely used semen parameters for assessing the fertility of the bull are based on sperm motility, concentration and sperm morphology. Although these parameters are basic and important for analyzing the male fertility yet they have limited importance for fertility evaluation of breeding bulls (Blasco, 1984). So there is a need of some viability test that can define or quantify the life of the sperm. Sperm penetration tests both in estrual mucus as well as in synthetic medium give more relevant information regarding the fertilizing ability. The present study was planned to find out the correlation between sperm penetration in estrual mucus and polyacrylamide gel with different seminal attributes.

Four adult breeding buffalo bulls (Punjab Agricultural University, Ludhiana and cattle farm Bhattian, Khanna) were included in the study. Twelve ejaculates (three from each bull) in Artificial Vagina were collected for the study. The freshly collected semen sample was evaluated macroscopically as well as microscopically. The semen freezing was done using Tris Egg Yolk Glycerol extender using medium sized French straw as per the method of Cassou (1964) and Bindra (1991). Prior to sperm mucus penetration test each semen straw/sample was evaluated for post-thaw motility, live sperm count and sperm morphology.

Estrual mucus sample was collected from 19 normally cycling buffaloes under aseptic conditions and evaluated for colour, consistency, pH, arborization and bacterial growth. This mucus was used to conduct the sperm mucus penetration test (SMPT) with all the semen ejaculates of four bulls under study. An artificial synthetic media i.e. 2% polyacrylamide gel (PAG) was prepared as per method of Lorton *et al.* (1981). The PAG (2%) was also evaluated for physical characteristics i.e. colour, pH, spin barkeit and visible gelification. This gel was used to conduct the sperm penetration test with the same semen samples (ejaculates) of the same bulls as was done for mucus sample. Frozen semen samples of buffalo bulls were thawed at 37°C for 30 seconds and standard semen parameters and SPD values in both mucus and PAG (2%) were recorded for each bull. Semen parameters and sperm penetration distance in both estrual mucus and PAG (2%) were recorded and presented in Table 1. The mean post thaw motility for the bulls varied significantly between 51.7±1.70% to 66.7±1.70%. The live sperm count in bulls varied from 84.0±1.0 to 89.7±1.20% while the normal sperm count was from 84.3±0.67 to 88.7±1.67%. The mean SPD values in estrual mucus were from 21.0±0.76 mm/min. to 25.0±0.5 mm/min. (Table 1). The values varies significantly between the bulls ($P<0.05$). The correlation

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Table 1. Sperm penetration in relation to seminal characteristics of bulls in mucus and PAG (2%)

Post-thaw semen	Bull No.*			
Parameters	M-1290	BH-6703	BH-0210	BH-3875
Sperm motility[a] (%)	51.7±1.70	53.3±1.70	63.3±1.67	66.7±1.70
Live Sperm count[b] (%)	84.0±1.01	86.0±3.60	88.7±2.90	89.7±1.20
Normal Sperm count[b] (%)	84.3±0.67	86.3±1.45	90.0±1.52	88.7±1.67
SPD value in mucusa (mm/20 min.)	21.0±0.76	22.3±1.01	24.7±1.30	25.0±0.50
SPD value in PAG[a] (2%)(mm/20 min.)	19.8±0.17	20.8±0.91	21.8±1.09	23.8±0.60

*3 ejaculates per bull.
[a]Mean value for the parameter differ significantly between bulls
[b]Mean values for the parameter did not differ significantly between bulls

coefficients of SPD values with live sperm count, normal sperm count and post-thaw motility were found to be 0.800, 0.613 and 0.567, respectively (Table 2). The SPD values in mucus were found to have significant correlation with live sperm count and normal sperm count. However, post-thaw motility was non-significantly correlated with SPD values. Similar observations have also been recorded earlier by several workers (Sidhu *et al*, 1986, Okuda *et al*, 1988 and Dev *et al*, 1996). This clearly indicates that kinetics and quality of progression of spermatozoa in estrual mucus is more important than merely the percentage of spermatozoa (Keel and Webster, 1988).

A significant correlation (0.800, Table 2) was observed between live sperm count and SPD value. Similar results were recorded by Kaur (1986) and (Dev 1995). This indicate that only the live spermatozoa are able to penetrate the micelle of the estrual mucus effectively. Murase and Braun (1990) also suggested that adequate lateral head displacement is essential for spermatozoa penetration in estrual mucus. Similarly normal form of spermatozoa play crucial role in deterimining sperm penetration. A significant coefficient of 0.613 was observed between normal sperm count and SPD values. The present values were in agreement with the result of earlier studies David *et al*, 1979, Dev *et al.*, 1996).

Table 2. Correlation coefficients between post thawed seminal characteristics of semen and SPD values in mucus and PAG (2%)

Seminal characteristics	Correlation coefficient	
	Mucus	PAG (2%)
Sperm motility	0.567	0.747*
Live sperm count	0.800*	0.615*
Normal sperm count	0.613*	0.389

* P<0.05

The mean SPD values in PAG (2%) varied significantly between bulls19.8±0.17 to 23.8±0.60 mm. (Table 2). A significant +ve correlation (r, 0.615) was obtained between live sperm count and SPD values in PAG. Eggert kruse *et al.* (1993) reported a slightly lower correlation coefficient for human semen in PAG. Similar to estrual mucus a non-significant correlation coefficient of 0.389 was obtained between sperm penetration and no. of normal spermatozoa in PAG. Eggert kruse *et al.* (1993) reported a significant higher correlation regarding sperm ability to penetrate the synthetic medium (PAG) as well as cervical mucus, with correlation values decreasing with higher concentration of PAG. However, Goldstein *et al.* (1982) reported a lower correlation coefficient with cryopreserved human semen but almost similar correlation coefficient with fresh human semen.

The difference in value of correlation coefficients as reported by various authors might be due to the difference of species or type of extender used for cryopreservation or concentration of PAG used or there may be difference in methodology involved.

It may be concluded that PAG (2%) serves as a substitute for estrual mucus because quality of estrual mucus varies with donor and it is also to be collected freshly, whereas PAG can be prepared in laboratory and additionally the sperm visibility is better in PAG than in mucus.

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