The Indian Journal of Animal Reproduction; 25(1): 61-62; June 2004

SHORT COMMUNICATION

Polyacrylamide gel as a substitute of estrous mucus for sperm penetration test

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Received : February 1, 2002
Accepted : January 17, 2003

ABSTRACT

Bovine estrual mucus is used for sperm penetration studies. But, major constraints are variation in the quality of mucus giving inconsistent result. For batches of 1, 2 and 3% polyacrylamide gel were prepared and examined for their physical properties viz., transparency, spinnbarkeit, pH and visible gelification. Two percent PAG resembled bovine estrous mucus, while 1% had watery consistency and 3% was very thick. The average pH values for the three grades of PAG were around 8.6. It was concluded that 2% PAG being more alike to bovine estrual mucus, could be used for sperm penetration studies.

Key words : Bovine estrual mucus, polyacrylamide gel, physical properties

Sperm migration in female genital tract depends on sperm motility, genital tract contraction and biophysical characteristics of cervical mucus (Overstreet and Katz, 1977, Panchal, 1988). Chemical and physical changes in cervical mucus affect sperm transportation from the site of ejaculation to the site of fertilization (Moghissi, 1972; Zavos and Cohen, 1980). Sperm from human and cattle ejaculates migrate variable distance depending upon the source of mucus donor and the time for which it has been stored (Kummerfeld *et al.*, 1981). To overcome this variability, a media with properties similar to those of estrous mucus was needed. Therefore, polyacrylamide gel with different concentrations was studied for their physical properties. Synthetic migration media, polyacrylamide gel was prepared as per the method described by Lorton *et al.* (1981).

Solutions used

Solution A : *Acrylamide bisacrylamide solution* : Fresh solution was prepared by dissolving 30 gm of acrylamide and 0.8 gm of BIS in 100 ml of double distilled water.

Solution B : Tris [Tris(hydroxymethyl) amino methane] buffer: Tris (1.5 M) was dissolved in 1000 ml of double distilled water and pH was adjusted to 8.8 with HCl. It was stored in refrigerator at 4°C in tightly capper bottle.

Solution C : Phosphate buffer saline-glucose (PBS-glucose): Phosphate buffer saline with 1% glucose comprised of 100 mM NaCl, 2.68 mM KCl, 86 mM $NaH_2PO_4.H_2O$, 12.7 mM Na_2HPO_4, 55.5 mM Glucose. Double distilled water was added to make final volume 1000 ml. pH was adjusted to 6.8 with HCl.

Solution D : *Ammonium persulphate* : It was freshly prepared by dissolving 2 gm Ammonium persulphate in 100 ml double distilled water.

Preparation of 1% polyacrylamide gel

Sol. A	0.67 ml
Sol. B	5.00 ml
Double distilled water	20.00 ml

Preparation of 2% polyacrylamide gel

Sol. A	1.34 ml
Sol. B	5.00 ml
Double distilled water aa.	20.00 ml

Preparation of 3% polyacrylamide gel

Sol. A	2 ml
Sol. B	5 ml
Double distilled water aa.	20 ml

To each grade of PAG, 300 µl Sol. D and 10 µl TEMED was added and allowed to stand for 12 hrs at room temperature. PAG was poured into dialysis tubing (Average flat width 32 mm, average diameter 20 mm, capacity 100 ml/ft; Sigma Chemicals Co. St. Louis, USA). Dialysis tubing containing PAG

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was kept in a beaker having PBS-glucose (pH 6.8) and dialyzed at 4°C for 24 hrs. PBS-glucose was changed every 6 hrs.

Physical examination of polyacrylamide gel : 1,2 and 3% polyacrylamide gel were examined for their physical properties viz., transparency, spinnbarkeit, pH and visible gelification. Spinnbarkeit is the extent of gelification/viscosity measured by taking a drop of gel on index finger. The thumb was brought in contact and then spread in opposite direction. The distance between thumb and index finger where continuity of the gel broke was measured in mm. pH was recorded by pH meter. Visible gelification was described as absent, slight, moderate and complete gelification present on the bottom of vials containing PAG.

Four batches of 1, 2 and 3% polyacrylamide gel were examined for their physical properties viz., transparency, spinnbarkeit, pH and visible gelification. All the batches of PAG were transparent. The average spinnbarkeit values of 1, 2 and 3% were 0.00, 10.68±0.98 and 2.32±0.28 mm, respectively. Average spinnbarkeit value for 2% PAG was significantly ($P < 0.05$) higher than that of 1 and 3%. Singh (1999) reported comparable spinnbarkeit value (14.33±0.27 mm) for 2% PAG, however, Kaushal (1998) found slightly lower (6.66±0.89) value for the same grade of PAG than the present study. The average pH values for three grades of PAG were around 8.6, which is in accordance with the findings of Kaushal (1998) and Singh (1999). There was no visible gelification in 1% PAG. however, in 2 and 3% PAG slight to moderate gelification was present, respectively. Two percent PAG resembled bovine estrous mucus, while 1% had watery consistency and 3% was very thick. These results are in accordance with Kaushal (1998) and Singh (1999). Based upon these observations, it was concluded that 2% PAG have physical properties similar to bovine estrual mucus, hence it could be used for sperm penetration studies.

REFERENCES

Kaushal, G. (1998). A comparative study on sperm penetration in polyacrylamide gel and estrual mucus in buffaloes. M.V.Sc. thesis, Punjab Agricultural University, Ludhiana, India.

Kummerfeld, H.L., Lortan, S.P. and Foote, R.H. (1981). Relationship of spermatozoal migration in cervical mucus to bovine fertility. J. Andro., **2**: 103-107.

Lorton, S.P., Kummerfeld, H.I. and Foote, R.H. (1981). Polyacrylamide as a substitute for cervical mucus in sperm migration test. Fertil. Steril., **37**: 668.

Moghissi, K.S. (1972). The function of cervix in fertility. Fertil. Steril., **23**: 295-306.

Overstreet, J.W. and Katz, D.F. (1997). Sperm transport and selection in the female genital tract. In : *Development in Mammals II* (M. Johnson, ed.), North Holland Publishing Co., New York.

Pachal, M.T. (1988). Studies on repeat breeding buffaloes with reference to cervical mucus characters, immuno-infertility, progesterone assay and tratment trial. M.V.Sc. thesis, Gujarat Agricultural University, Anand, India

Singh, S. (1999). Assessment of spermatozoa penetrability through polyacrylamide gel column in fertile and subfertile buffalo semen. M.V.Sc. thesis, Punjab Agricultural University, Ludhiana, India.

Zavos, P.M. and Cohen, M.R. (1980). The pH of cervical and post coital test. Fertil. Steril., **34**: 234-237.