IN-VITRO CAPACITATION OF BOVINE SPERMATOZOA BY MEASURING PENETRATING POTENTIAL*

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ABSTRACT

Penetrating potential of bovine spermatozoa, capacitated in vitro, was studied in terms of sperm penetration distance (SPD) to determine the suitable combination of caffeine concentration and incubation period for optimum capacitation. The capacitation was induced in sperm TALP (Tyrode'S albumin, lactate and pyruvate) medium containing 0, 5, 10 and 20 mM caffeine at 39 °C for 0, 30, 60 and 90 min. The capacitated spermatozoa were subjected to penetration in 2% polyacrylamide gel and SPD was measured. The maximum SPD of 19.35±0.50 mm /hr was recorded at 20mM caffeine with 90 min incubation period indicating this period and concentration combination as the most effective enhancer of in vitro capacitation of bovine spermatozoa.

Keywords: Buffalo spermatozoa, Caffeine, Capacitation, Gel penetration

INTRODUCTION

Mammalian spermatozoa have to spend some time in female reproductive tract for some changes called capacitation, which makes them able to fertilize the ova (Chang, 1951; Gordon, 1994: Harrison, 1996). They are actively motile, although, they are unable to fertilize the egg (Austin, 1960). Level of in vitro capacitation decides the success of in vitro fertilization and hence, embryo production in vitro.

Caffeine has been used to induce in vitro capacitation of bovine spermatozoa (Ax *et al.*, 1985; Parish *et al.*, 1989). However, different concentrations of caffeine and incubation periods have been reported with variable success (El -Mcnoufy *et al.*, 1986; Fraser, 1979). Further, in vitro capacitation is usually evaluated on the basis of hyper motility, head to head agglutination and acrosome reaction. Penetration of ovum by sperm during fertilization is a crucial event. Hence, study of penetrating potential of capacitated spermatozoa would be more logical. However, information on penetrating potential of capacitated spermatozoa is scarce. The sperm penetration in gel and mucus was significantly and positively correlated with post-thaw motility and acrosome integrity. The acrosome integrity has a more significant contribution. The correlation established with sperm penetration in gel was very similar to that of sperm penetration in mucus (Kumar *et al.*, 2001). Sperm migration in cervical mucus is related to the ability of spermatozoa to colonize the oviduct and to fertilize matured oocytes in vivo (Cox *et al.*, 2002). A material with sperm migration characteristics similar to those of cervical mucus might solve the problem. Further, it would be more beneficial if the material could be obtained or prepared simply with uniform and stable characteristics. Polyacrylamide gel (2%) is such a material, which resembles the normal estrual mucus and sperm migration distance reflects the migration capabilities in female genital tract (Kaushal, 1998).

Therefore, penetrating potential of in vitro capacitated spermatozoa of crossbred bull through polyacrylamide gel was studied in the present experiment to determine the suitable combination of caffeine concentration and incubation period for optimum level of capacitation in vitro.

MATERIALS AND METHODS

Ejaculated semen, from two crossbred bulls, was evaluated and deep frozen if found suitable. Deep frozen semen of satisfactory quality was processed for in vitro capacitation.

Actively motile frozen spermatozoa were separated by swim up method and 2 ml sperm suspension was mixed with 4 ml sperm TALP (Tyrode's albumin, lactate

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and pyruvate) in a test tube for washing. The mixture was centrifuged at 200 g for 10 minutes. Supernatant was decanted; pellet was resuspended in 2 ml of sperm TALP and centrifuged, as earlier. The supernatant was decanted and the washed spermatozoa were re-suspended in 2 ml sperm TALP.

Now, 100 µl sperm of washed sperm suspension, obtained as above, was layered down in 16 test tubes (4 tubes each of 0, 5, 10 and 20 mM caffeine containing 1 ml of sperm TALP). All the tubes were incubated at 39°C for 90 min to induce capacitation. Penetrating potential of spermatozoa was determined at 0, 30, 60 and 90 min of incubation for all concentration groups, employing polyacrylamide gel penetration test (Kumar *et al.*, 2003).

Polyacrylamide gel (2%) was taken in the capillary tubes and then one end of the tubes was plugged with haemoseal. One hundred micro liters of incubated semen samples was taken at each period from all concentration groups in the vials separately. The capillary tubes were fitted in the inverted position in the vials with open end contacting the semen. The vials were incubated for one hour at 37°C. Thereafter, the capillary tubes were removed from the vials, placed on the microscopic slides with attached graph paper and examined under phase contrast microscope (200 x). The tubes were scanned from the end which had been in the sperm reservoir to the most distant point having vanguard spermatozoa. This distance measured in millimeters indicated the penetrating ability of spermatozoa. The experiment was replicated for 20 times (10 replicates/bull). Data were statistically analyzed using two-factor completely randomized design (Snedecorand Cochran, 1967).

RESULTS AND DISCUSSION

The observation on sperm hyper motility and acrosome reaction revealed that the capacitation progressed well. Sperm penetration distance was 16.80±0.34 mm/hr with the highest concentration (20mM) of caffeine after 30 minutes of incubation. The observations after 60 and 90 minutes of the incubation period were 18.60±0.40 and 19.35±0.50 mm/hr, respectively again with the highest concentration. Similarly, Kumar *et al.*, (2003) reported the sperm penetration distance of frozen-thawed bovine spermatozoa in 2% Polyacrylamide gel to be 16.46±1.29 mm/hr, which increased to 20.67±1.55 mm/hr after sephadex filtration. It is thus discernible that any factor, which increases the proportion of active and motile spermatozoa, would be responsible for increase in the sperm penetration distance in the polyacrylamide gel, which is also evident from the above finding of the present investigation.

There was a significant ($P < 0.01$) difference in SPD among all the concentrations, at each of 30, 60 and 90 min. of incubation period. The SPD with all the concentrations at 30 and 60 min. of incubation periods increased significantly to their previous complimentary incubation periods ($P < 0.01$). It is thus discerned that the increase in the concentration of caffeine and incubation period except beyond 60 min. had a significant effect on sperm penetration distance in 2% polyacrylamide gel during capacitation. It may be concluded that the increase in the hyperactive spermatozoa during capacitation may be responsible for the increase in the penetration distance of spermatozoa in 2% polyacrylamide gel.

The present conclusion was well supported by Marquez and Suarez (2004) and Gardon *et al.*, (2001) who reported that 10 mM and 20mM of caffeine respectively was sufficient to induce hyper activation of bovine spermatozoa incubated for IVC.

Parrish *et al.*, (1989) reported that caffeine, a phosphodiesterase inhibitor, significantly induces capacitation of bovine spermatozoa in vitro at a concentration of 10 mM. It causes intracellular accumulation of calcium and also stimulates protein kinase which regulates overall calcium influx and thus, the motility and penetrating ability of spermatozoa through polyacrylamide gel.

It was concluded that the maximum SPD of 19.35±0.50 mm/hr was recorded at 20 mM caffeine with 90 min incubation period indicating this period and concentration combination as the most effective enhancer of in vitro capacitation of bovine spermatozoa.

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