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Short Communication

STUDY ON CRYOPRESERVATION OF PRIMARY GOAT KIDNEY CELL AND ITS SUSCEPTIBILITY TO INFECTION *IN VITRO*

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ABSTRACT: Primary goat kidney cells are used for isolation, propagation and production of vaccine against goat pox virus. Availability of kid at desired time is a major constraint for uninterrupted vaccine production. Keeping this in mind, this study was designed to standardize a method of preservation of primary goat kidney cell for a long period. In the present study, culture, maintenance and preservation of primary and secondary goat kidney cell were conducted and was observed that primary goat kidney cell can be successfully preserved in liquid nitrogen (-196^{0}C) for a period of one year. After each revival, the cryo-preserved cell could be propagated and the confluent monolayer of primary kid kidney cells was given infection with Goat Pox virus at 0.1 m.o.i. based on predetermined $TCID_{50}$ of virus. The susceptibility of revived cells to Goat Pox virus infection was studied by characteristic cytopathic effect and the identity of the viral DNA in infected cell lysate was confirmed by PCR upto the cells of 9th subculture after revival.

Key words: Cryopreservation, Primary goat kidney cell, Susceptibility, Infection, PCR, *In vitro.*

A number of cell lines are in use for isolation and propagation of virus *in vitro.* Use of Vero cell for isolation of PPRV (Nanda *et al.*, 1996), BHK21 for Blue Tongue Virus (Ramesh Babu *et al.*, 1992), PK15 for Classical Swine Fever Virus (Dhar *et al.*, 2008) is common. Primary cell cultures have been used for propagation of different viruses. Lamb kidney culture had been used for isolation of field viruses like Peste des petits ruminants virus, Blue tongue virus and Sheep and Goat pox virus (Taylor and Abegunde 1979, OIE manual 1996). Attempts have been made for preservation of primary cell culture by various workers. Parthiban *et al.* (2005) preserved chicken embryo fibroblast cells at 4^{0}C for 15 days in storage medium. Lamb kidney cells preserved in vapour phase of liquid nitrogen could be revived, propagated and used successfully for propagation of virus (Govindarajan *et al.*, 2008). Primary goat kidney cell is used for *in vitro* propagation of Goat Pox virus and vaccine production. In this present study attempts have been made to cryopreserve the primary goat kidney cell in liquid nitrogen with a target to provide the cell for production of vaccine uninterruptedly.

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Materials used: Trypsin (0.25%) in calcium and magnesium free PBS 10 X con (Himedia), Streptomycin (Sigma) 100 mg / lit., Penicillin 60 mg / lit. of media, EMEM(Sigma) with glutamine and Phenol red,
FBS (Himedia), DMSO (Sigma), sterile cell culture flasks 25 cm^2 and 75 cm^2 (TPP), cryovials (TPP), versene (sigma), glucose (Sigma), PBS (Sigma).

Primary culture: Primary culture was done according to the method described in FAO, Animal Production and Health paper 118 (1994) with slight modification. Goat kidney was collected from one day old kid (prior permission was obtained from the competent authority of the Institution before carrying out the study) from a mother not vaccinated against goat pox. After sacrificing the kid whole kidney along with fascia and capsule was collected aseptically in a large sized beaker containing PBS with antibiotic (Streptomycin 100 mg/lt. and Penicillin 60 mg/lt.). After collection, kidney was transferred into a petridish containing PBS with antibiotic, where fascia and capsule were removed and washed several times. The Cortical portion was cut into small pieces, kept in a narrow mouthed beaker containing PBS and minced into further small pieces. Cut tissues were then washed thrice with PBS and once with trypsin. Trypsinization of tissues was done with 0.25% trypsin for 15 minutes. Supernatant was discarded. Trypsinization continued with fresh trypsin solution for another 30 minutes. Dispersed cells were harvested through a sterile muslin cloth filter in a flask containing fetal bovine serum (FBS) and kept at 4^0C. Fresh trypsin was poured and process of trypsinisation was continued for two to three times. Entire process of trypsinisation was performed at 37^0C.The harvested cell was centrifuged at 1500 rpm for 15 minutes in a cooling centrifuge. Cell pellet was resuspended in the growth medium (EMEM) without serum and again centrifuged. After two such washing the cells were finally resuspended in a measured volume of growth medium (EMEM containing 10% FBS) and dispensed in a number of 25 cm^2 tissue culture flask at the rate of 8 ml each and incubated at 37°C.

Propagation of Primary cell: Propagation of the primary cell monolayer was done by trypsinising with Trypsin-Versine solution. Cells were distributed at 1:3 ratio and nine such subcultures were performed consecutively.

Cryopreservation of cells: Cells were harvested by trypsinisation and pooled in EMEM with 10% FBS, centrifuged to make cell pellet which was finally resuspended in cryopreservation medium (EMEM, 20% FBS and 10% DMSO). Cell suspension was distributed in cryovial @ 1 ml and cooling in step down process@ 1^0C / min., kept at -80^0C overnight, at LN_2 Vapour for 6 hours and ultimately immersed in liquid nitrogen (-196^0C) for long term storage.

Revival of cryopreserved cell: Cryopreserved cells taken out from LN_2 thawed at 37^0C, suspended in 8 ml growth medium (EMEM+15% FBS) and incubated at 37^0C. Revived cells subcultured up to 7^{th} passage successfully, thereafter growth rate declined. Revival was carried out at monthly interval up to 1 year.

Susceptibility to virus infection: Goat Pox vaccine virus (KSGP 0240 Strain) was inoculated at 0.1 m.o.i. onto the cell monolayer

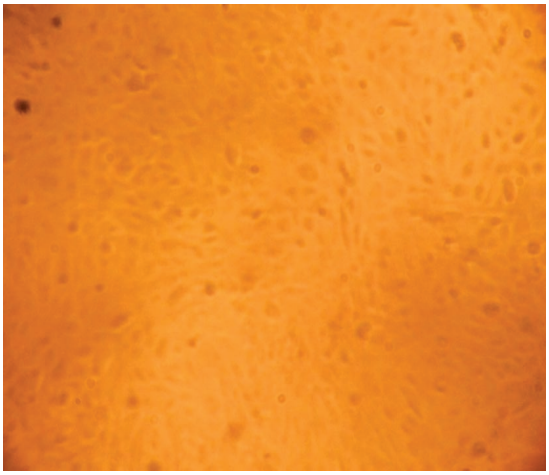

Fig.1: Pre-infection stage.

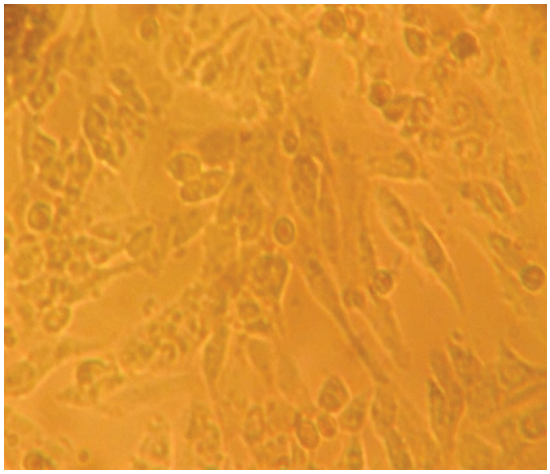

Fig. 2: Post-infection stage (2nd day).

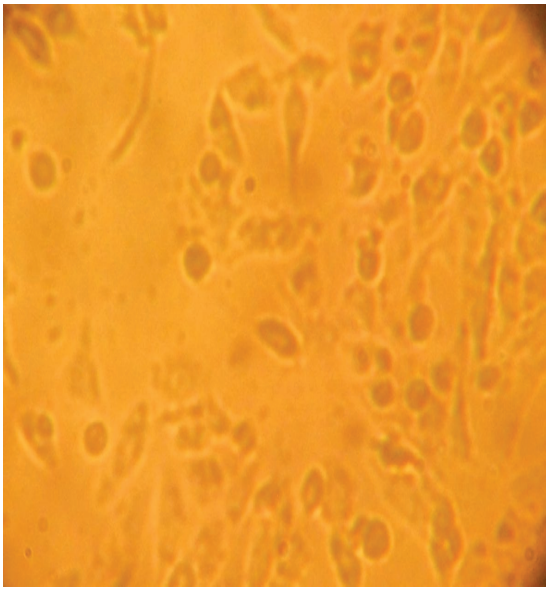

Fig.3: Post-infection stage (5th day).

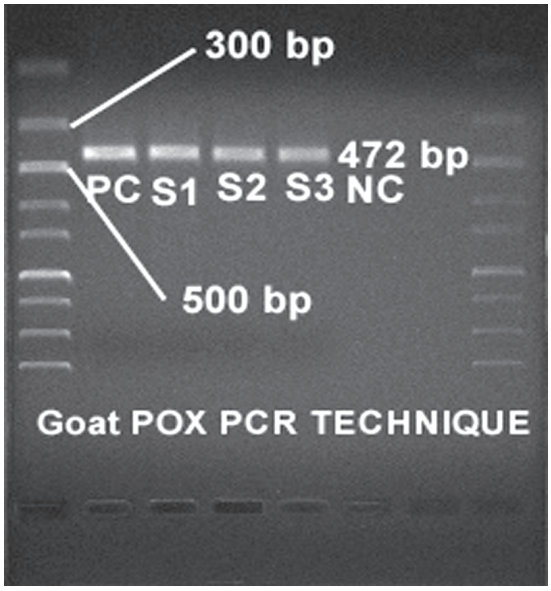

Fig. 4: PCR observation.

of different passage level and was observed for characteristic CPE up to 9th passage cell of Primary as well as cell under cryopreservation upto a period of 1 year. Aliquots of Goat Pox virus infected cell showing 80% CPE were collected at different time intervals and preserved at -70^{0}C after three cycle of freezing-thawing. Viral DNA was subjected to PCR.

PCR test:

DNA Extraction procedure: Viral DNA from the aliquots of 2nd Day, 3rd day and 5th day post

infected cell culture fluid was purified using Quiagen® DNA mini kit as per the manufacturer's protocol and subjected to PCR as per the method employed by Ireland and Binepal (1998) with slight modification.

Primer: PCR primers for Capripox fusion gene, employed by Ireland and Binepal (1998) having following sequence Forward Primer 5'ATGGACAGAGCTTTATCA 3' and Reverse Primer 5' TCATAGTGTTGTACTTCG 3' (GCC Biotech, Kolkata) were used.

PCR technique: The PCR technique was carried out using the above mentioned Primers and Maxima Hot Start 2X Master mix (Fermentus®). Briefly, 25 µl of 2X Master mix, 0.5 µM Forward and Reverse primer and 5µl of template purified as described was added to sterile nuclease free distilled water to make final reaction volume of 50 µl. Thermal cycling was carried out at 95^0 C for 1 minute x 1 cycle, followed by 35 cycles of 94^0 C for 30 Seconds, 55^0 C for 30 Seconds, 72^0 C for 30 Seconds and a final elongation step of 72^0 C for 10 minutes using Applied Biosystem 9700 Thermal Cycler. 10 µl of PCR product along with 2 µl of 6X Orange Gel loading dye(Fermentus®) was loaded per well of 1% agarose gel containing ethidium bromide(Sigma) and the electrophoresis was run in TAE buffer at 100 volt for 1 hour along with 100 bp ladder (Gene ruler, Fermentas) . After electrophoresis the gel was analyzed using Gel documentation system (Bio Rad).

A confluent monolayer of primary goat kidney cell was obtained after 4^{th} days incubation (Fig.1). Though after 7^{th} subculture, growth rate diminished but cellular morphology was unchanged up to 9^{th} passage. Cryopreservation was successful up to 1 year as the cells preserved in liquid nitrogen were satisfactorily revived and propagated. Cell monolayer formed from the revived cell could be infected successfully by Goat Pox vaccine virus. Characteristic CPE was observed up to 9^{th} passage cell of Primary as well as cell under cryopreservation up to 1 year (Fig. 2, 3). Infectivity of the specific virus was confirmed by PCR. Size of the PCR product was 472 bp (Fig.4) as expected in all the aliquots of infected cell lysate and also in accordance with the observation by Ireland and Binepal (1998).

Here the PCR has been carried out to establish the identity of Viral DNA responsible for cytopathic effect in infected cell. As the expected PCR product size specific for Goat Pox viral DNA amplified with specific set of primers for fusion gene of Goat pox virus has been observed by analysis of ethidium bromide stained bands after gel electrophoresis, it can concluded that CPE was due to only Goat Pox virus and not for any extraneous agent.

Though primary lamb kidney cells have been found more sensitive for propagation of viruses, cell culture laboratories prefer established cell lines due to the convenience of maintenance and preservation. Much study reports are not available regarding the methods of maintenance and preservation for primary cells to meet up continuous need of primary cell in a vaccine production laboratory. So attempts were made to standerise a method for long time preservation. Storage of chicken embryo fibroblast cells at 4^0C by Parthiban *et al.* (2005) or preservation lamb kidney primary cells at LN_2 vapour by Govindarajan *et al.* (2008) was limited to a period of 15 days and 6 month respectively.

In this study, effective preservation of

Primary goat kidney cell in liquid nitrogen (-196^{0}C) up to one year (period of study) pave the way of cell preservation one step ahead. Moreover, infectivity of revived cell was evaluated by propagation of Goat pox virus in the cell even after repeated 9th subculture. Confirmation of virus in infected cell lysate by PCR establishes the study for wide acceptance. From this study, it can be concluded that a single primary cell culture from goat kidney can provide desired primary cells to the laboratory throughout the year as and when required avoiding the troubles of several primary cultures in a year.

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REFERENCES

Dhar P, Rai A, Verma R (2008) Adaptation of classical swine fever virus (lapinized strain) in PK-15 cells and confirmation by reverse transcription-polymerase chain reaction (RT-PCR) and fluorescent antibody technique (FAT). Indian J Anim Scs 78(2): 135-137.

FAO Animal Production and Health Paper 118 (1994) Quality control testing of rinderpest cell culture vaccine. Standard operating procedures. 1-18.

Govindarajan R, Manoharan S, Purushothaman V, Chandran NDJ, Prabhakar TG (2008) Observations on the maintenance of *in vitro* primary and secondary lamb kidney cells. Tamilnadu J Vet Anim Scs 4(6): 215-218.

Ireland DC, Binepal YS (1998) Improved detection of capripox virus in biopsy samples by PCR. J Virol Meth 74: 1-7.

Nanda YP, Chatterjee A, Purohit AK, Diallo A, Innui K, et al. (1996) The isolation of Peste des petits ruminants virus from North India. Vet Microbiol 51: 207-216.

OIE manual (1996) Manual of standards for vaccines and diagnostics. Chapter 2.1.10. Sheep pox and Goat pox. 119-127.

Parthiban M, Manoharan S, Wilson Aruni A, Daniel Joy Chandran N, Koteeswaran A (2005) A simplified method for maintenance of chicken embryo fibroblast culture. Asian J Microbiol Biotech Env Sci 7(2): 251-253.

Ramesh Babu NG, Byregowda BM, Bragitha AJ, Gopal T (1992) Isolation and characterization of blue tongue virus from sheep. Indian Vet J 69: 1071-1074.

Taylor WP, Abegunde A (1979) The isolation of Peste des petits ruminants virus from Nigerian sheep and goats. Res Vet Sci 26: 94-96.

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