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# *Invitro* culture of mesenchymal stem cells from ovine amniotic fluid cells

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**ABSTRACT**

Fetal tissue engineering has emerged as a promising concept in surgical reconstitution of certain birth defects. Utilizing minimally invasive techniques, fetal tissue has been harvested, cultured and manipulated *invitro* and the resulting autologous cellular bioprosthesis is used for implantation shortly after birth. Thus far, this concept has been used sucessfully in animal experiments for the reconstruction of bladder, skin and diaphragmatic deformities. These preliminary reports are promising and harvesting fetal tissue and its culture could be a best source of stem cells with remarkable plasticity (Temple,2001).

**Key words:** Ovine amniotic, mesenchymal stem cells, *In vitro* culture.

## INTRODUCTION

Recently attention to amniotic epithelial cells has been directed as a source of cells for tissue replacement therapies (Sakuragawa *et al*.,1996). These cells may have a potential to differentiate among such various organs as the brain, heart and liver. It has been reported that the fetal mesenchymal cells can be isolated consistantly from the amniotic fluid (Amia kaviani etal.,2001).Hence it has been proposed to culture the amniotic fluid cells from ovine under *invitro* conditions for their applications in tissue engineering.

Ovine amniotic fluid were collected in sterile conditions from the fetuses obtained from Perambur slaughterhouse, Chennai (transported to the laboratory within 30 to 60 minutes of slaughter). The fluid was processed for isolation of amniotic cells by a two-step protocol (Tsai *et al*., 2006). Primary amniocyte cultures were set up using standard protocol. The nonadherent pelleted cells were washed two to three times in PBS. The cell pellet was resuspended in sterile medium amniotic fluid cells in the supernatant medium were collected, centrifuged and then plated with alpha modified minimum essential medium supplemented with fetal bovine serum and 20ng/ml supplementation of basic fibroblast growth factor, and plated in tissue culture plates at a concentration of $2X10^{6}$ cells per ml and incubated in $37^{0}$ C at 5% $CO_2$. The cells could form a monolayer in 24-48 hours. The cells were subcultured and maintained using 0.25% trypsin EDTA treatment with a split ratio of 1:3. The adherent cells could form a mono layer revealing morphologies of different cell types viz., endodermal, mesodermal and epidermal derviates (Fig). This finding could correlate well with the reports on the use of fetal membranes as stem cell sources such as, Priest *et al.,* (1997) Chan *et al*., (2005) and Tsai *et al.,* (2006).

Thus, it is revealed that the fetal sac fluid could be used as a source of obtaining stem cells and can support the findings that the cultured amniotic cells were used as source for transplantation therapies (Adinolfi *et al*., 1982). These cells can produce substantial quantities of enzymes lacking in more common enzyme disorders as reported by Akle *et al*.,(1981), can produce enzymes such as adenosine deaminase which are of diagnostic and therapeutic value. The implantation of either sheets of amniotic epithelial cells or cells cultured i*nvitro* has many advantages

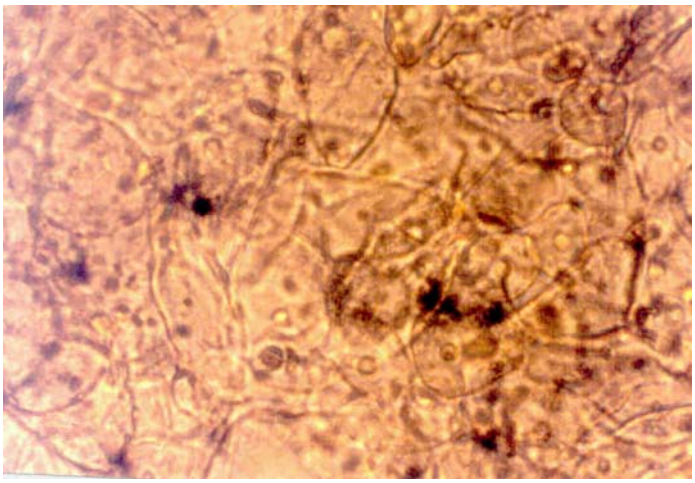

**Fig. -1: Monolayer of Amniotic fluid cells 40X**

over the use of bone marrow or fibroblast transplants with minimal graft versus host reaction (Adenolfi *et al*.,1982).The studies on the hematopoietic fate adopted by adult neural stem cells (Temple,2001) reveals that the amniotic epithelial cells could be a positive source for transplantation therapies as these cells have surface markers for neuronal stem cells (Sakuragawa *et al*.,1997). Hence, further research on the characterization of amniotic epithelial cells and their use for transplantation following genetic engineering could make the cells suitable for gene therapy.

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