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Original article

Evaluation the bovine oviduct epithelial cells culture in monolayer system

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ABSTRACT

The oviduct is an important site in reproduction, such as fertilization and early embryonic development. In this study bovine oviduct epithelial cells (BOEC) isolated and cultured in monolayer system to evaluate the proliferation and viability of cells in quality manner before and after two time passages. The epithelial cells isolated mechanically from oviduct tissue in sterile condition. Isolated cells floated in culture medium, fetal bovine serum and antibiotics in a T-24 culture flask. Two days after seeding have been observed that cells adhered to flask surface and are proliferating. The rate of cell proliferation was well. After one week the cells covered the flask surface and were confluent. Then the cells isolated from flask by trypsin-EDTA enzyme and passage into 4 flasks. Cells cover the flasks in 10 days and passage into 12 flasks. In the second passage the cells was confluent in 12 days. We observed that in first culture there was a little dead cell during the culture and cells proliferation rate was high. After each passage the number of dead cells in flasks increased and cells proliferation rate in each passage decreased.

1. Introduction

Macroscopically, the oviduct in cow is a simple organ about 21-28 cm long and is divided into 3 areas: the infundibulum, the ampulla and the isthmus (Ellington, 1991). Oviduct is an organ which has multiple roles in female bovine reproductive tract. Several researchers of animal reproductive system focus their researches on this organ. Oviduct is an organ that has a role in maturation and female and male gamete transportation and also provides appropriate condition for fertility and fetus growth and fetus transportation in uterus. Specific and physiologic reaction between gamete and fetus with oviduct epithelial cells is important. Epithelium increases the viability and mobility of spermatozoa and balances the sperm capacitating, also boosting the ovule maturation quality (Schoen et al., 2008). Therefore, it seems likely that oviductal epithelial cells are deeply involved in the reproductive and developmental events that occur in the oviduct. Bovine oviduct cell monolayers have been employed as an in vitro co-culture system for InVitro produced bovine embryos (Xu et al. 1992; Myers et al. 1994); however, the rates of embryonic development are still lower than in vivo. This phenomenon may be due to dedifferentiation processes, loss of morphological and functional properties of oviduct cells occurring under standard culture conditions (Joshi 1988; Hishinuma et al. 1989; Walter 1995). The use of BOEC co-culture systems has improved embryonic development in nearly all the studies conducted. In addition, interaction of bovine spermatozoa with BOEC, in a similar manner to that observed for spermatozoa in vivo, induced specific changes in sperm capacitation and consequently improved the fertilizing capacity of bovine spermatozoa in vitro (Abe and Hoshi, 1997). Also BOEC can be use for experiments with the aim of evaluating the genes expression, interactions between oviduct epithelial cells and gametes or embryos. These experiments cannot be studied in vivo. Therefore, model systems are needed which mimic in vivo conditions most closely. In this way, a study conducted to evaluate the cell growth, proliferation and viability by means of bovine oviduct epithelial in monolayer cells culture before and after two times passages.

2. Materials and methods

2.1. Materials

Dulbecco's Modified Eagle's Medium (DMEM), phosphate buffer solution (PBS), 0.25% trypsin-EDTA solution, Penicillin, Erythromycin, were purchased from Sigma–Aldrich Co, USA. Fetal bovine serum was obtained from Invitrogen, USA.

2.2. Cell culture

Oviduct was obtained from pregnant cow that slaughtered at an abattoir. A specimen of 4×4 cm of oviduct tissue cut out in sterile condition and after washing with PBS, samples were placed in falcon containing 9 mL DMEM and 1mL FBS medium and transferred to the lab on ice. For establishment of monolayer cultures, the speciment were squeezed into a 35-mm Petri dish. Oviduct tissue sample has been washed with phosphate buffer saline (PBS) and culture medium two times. The epithelial cells were isolated mechanically by scratching the both surface of tissue with plastic scraper. The pure oviduct epithelial cells were suspended in T-25 culture flask containing of 4 ml culture medium (Gibco), 10% fetal bovine serum (FBS) (Sigma) and 50 U/ml Penicillin-Erythromycin. By 48 h, cells observed under invert microscope (AUSTRIA micros) and approximately 20% of the surface of flask was covered by adherent cells. The cultures were maintained by removal of the old medium and addition of 4 ml of fresh culture medium every 48 h. By week 1 the monolayer was confluent.

2.3. First passage of cells

Culture Medium removed from flask by sterile pipette. 200 µl Trypsin-EDTA added to cells and incubated for 5 minute to detach from floor of flask and after that 2 ml FBS added to deactivate the enzyme. The mixture transferred to microtube and centrifuged (Heraeus-Biofuge) in 106 × g for 5 minute to sediment. The liquids removed and medium added to cells and pipette to float the cells in the medium. Then cells passage into 4 culture flasks. After 8 days the cells covered the whole surface.

2.4. Second passage of cells

The cells of each treatment detached in the same method described in above and passage into 3 flasks as a second passage.

3. Results and Discussion

By culturing the isolated cells from oviduct tissue in monolayer culture, a lot of cells after one or two days were dead. These dead cells were floated in culture medium and could be observed in the shape of no color beads (crystalloid) under the invert microscope. In the same time some of the cells attached to flask surface and started to proliferation. Initially the cell growth was so slow. For prevention of alive cells toxicity due to dead and destroyed cells, the primary culture medium substituted with fresh one. After that cell growth rate increased. The cells growth and proliferation developed and after one week covered the whole of flask surface. When the cells proliferated more and more, the intensity of cells increased and cells seems to be smaller.

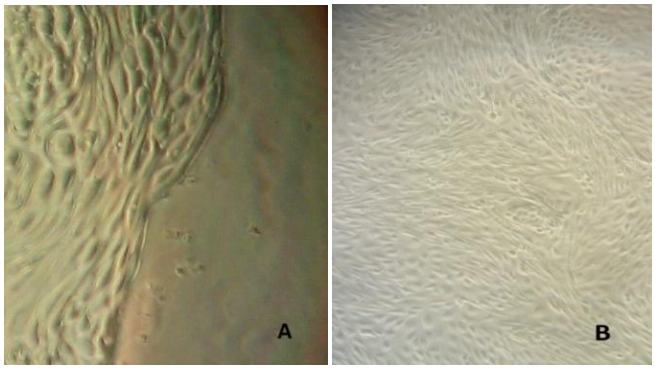

Fig.1. Images of bovine oviduct epithelial cell culture. A: cells cover a little part of flask surface (magnification 200×). B: since the cells cover the whole of flask surface and they are confluent (magnification 100×).

Cells covered the whole surface in one week. Cells accounted to obtain estimated number of it in the flask. Some of the cells detached from flask and colored by Trypan-blue and counted on Neubauer lam under invert microscope. Cells numbers approximately were 45×105. After that flasks treated with Trypsin-EDTA and cells had passaged into 4 flasks. In fact, these cells were primary culture and attached stronger to surface rather than cell lines. With treating by Trypsin approximately 50% of the cells isolated from surface. The passaged cells started to proliferation till after 10 days were confluent, followed by that cells passaged into 12 flasks. In second passage the cells were confluent in 12 days. Therefore the cells proliferation rate decreased after each passage.

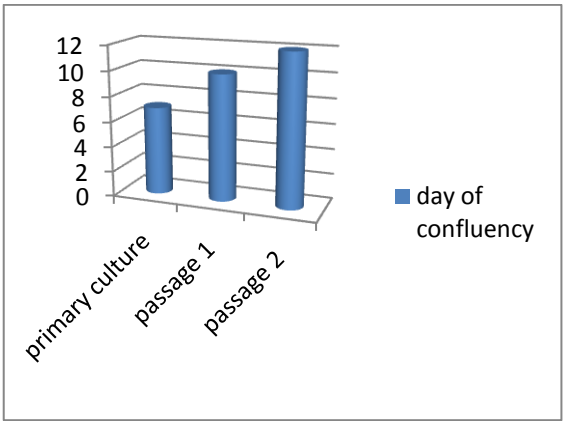

Fig.2. In primary culture, first and second passage, cells were confluent in 7, 10 and 12 days respectively. Effect of number of passage on cell growth showed on diagram. In each passage, the day of confluence increased.

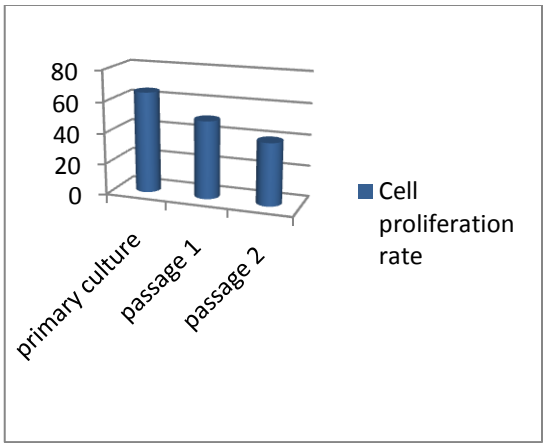

Fig.3. Cell proliferation rate in first and second passage decreased due to using of trypsin-EDTA enzyme to isolate attached cells from flask surface and difference of rate is illustrated on diagram. In primary culture, the cell proliferation rate is desirable but in each passage the rate decreased.

In primary culture in day one and two the amounts of dead cells were high but after media substitution the dead cells removed and adherent cells remained. In continue the number of dead cell decreased. In the first and second passage, the number of dead cells increased.

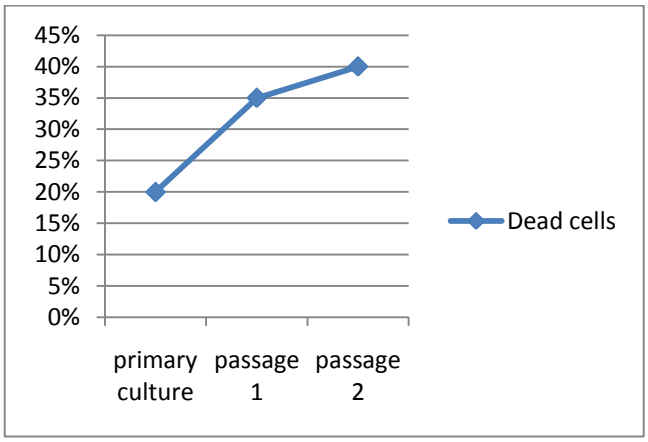

Fig. 4. In primary culture, passage one and two, the dead cells were approximately 20%, 35% and 40% of whole cells respectively. Linear diagram shows that the number of dead cells increased after each passage.

A considerable influence of various support materials on the morphology and functionality has been documented for cultured epithelial (Patrone et al. 1992) or endothelial cells (Villars et al. 1996). To study the relation of mammalian oviduct with secretory activity, influence of hormones and growth factors and to investigate mechanisms of embryo-maternal communication, a cell culture system is needed resembling the oviduct epithelium in vivo most closely (Reischl et al., 1999). Because of some difficulties and challenges to conduct these experiments In Vivo conditions it should have taken place In Vitro in monolayer or 3dimension culture systems.

Dead and floated cells in culture medium would be a toxicity factor and harmful for alive cells, therefore it is important to consider in culture systems and should be remove immediately. In this study we observed that the number of dead cells in each passage increased. It might be because of using trypsin-EDTA enzyme in each passage to detach the cells. Also cells become weak and proliferation rate decreased. In each passage take longer to fully cover the flask.

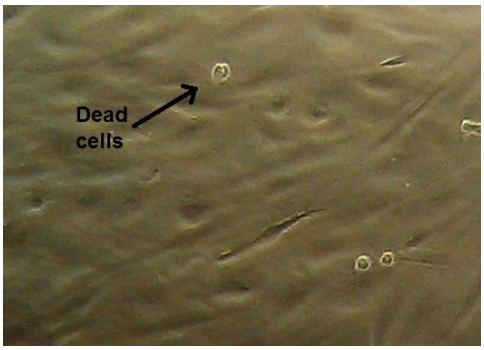

Fig.5. Dead cells in bovine epithelial cell culture medium (magnification 200×)

4. Conclusion

In conclusion in monolayer culture by passaging the bovine pure oviduct epithelial cells, it would lose its initial persistence and potency. The proliferation rate had decreased and number of dead cells had increased after first and second passage.

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