

Isolation, culture and characterization of endometrial stromal cells in buffalo

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Elucidation of the functions of endometrial stromal cells is important to understand the basic information of implantation and pregnancy in mammals including buffalo. Although both epithelial and stromal cells can produce PGF_{2α}, different morphological and physiological properties have been reported in cattle (Skarzynski *et al.* 2000). Stromal cells produce mainly PGE₂ whereas epithelial cells preferentially produce PGF_{2α}. However, to investigate the function of endometrial cells it is essential to isolate pure population of stromal cells. Moreover, it is difficult to separate a population of endometrial cells of one type that is not contaminated with endometrial cells of another type or with other cells present in buffalo uterus such as fibroblasts or vascular endothelial cells. The objective of the present study was to establish a system for the isolation, culture and characterization of primary culture of stromal cells isolated from buffalo uterus.

Buffalo uteri were collected from the local abattoir immediately after slaughter and transported to the laboratory on ice. The stages of estrous cycle were determined by colour, vasculature, size and consistency of corpus luteum (Verma-Kumar *et al.* 2004). Uteri of mid estrous cycle (days 5–10 of the cycle) were used in this study. The stromal cells were separated from buffalo endometrium using a modification of procedure described by Yamauchi *et al.* (2001). Briefly, endometrial tissues were washed in PBS three-times and digested in RPMI-1640 medium containing 0.1% collagenase at 37°C for 1 hr. The cell suspension was filtered through 70 mM strainer to remove the undissociated tissue fragments. The filtrate was washed 3-times by centrifugation at 600 g for 10 min with HBBS medium supplemented with gentamycin and 0.1% BSA. The number of viable cells that excluded trypan blue was counted using a haemocytometer and were cultured in RPMI-1640 medium containing 10% bovine foetal serum. Sixteen hours after the primary culture, the contaminating epithelial cells were eliminated by treatment with 0.05% trypsin solution with 0.53 mM EDTA

and the remaining stromal cells were cultured in RPMI 1640 medium at 38.5°C in presence of 5% CO₂ for 7 days. The medium was changed every 2–3 days until the confluency was reached. The cells were washed three-times with DPBS and harvested by centrifugation at 500 g for 15 min on day 3, 5 and 7 of culture. Growth pattern was monitored by measuring the levels of protein by folin-phenol reagent (Lowry *et al.* 1951) and of DNA by diphenylamine method (Burton *et al.* 1956) in the cell pellet. Data were analyzed by repeated measures analysis of variance (ANOVA) to find out the statistical significance of differences using statistical software (SPSS 16.0).

The stromal cells obtained after digestion with collagenase consisted of a mixture of fibroblasts, erythrocytes and epithelial cells. The stromal cells attached to culture dishes within 36–48 h after plating and other contaminating cells such as erythrocytes and epithelial cells were removed by replacement of the culture medium. In primary culture, stromal cells exhibited flat, fibroblastic and spindle shaped (Fig. 1) and showed streaming at the stage of confluence (Fig. 2). The profiling of protein and DNA serve as a valuable indicator of growth pattern of stromal cells in culture. Stromal cells grew rapidly at first (up to day 5 of culture) but slowed down on day 7 as the culture approached confluence. Protein concentrations increased from day 3 to day 5 ($P = 0.014$; $n = 6$) and further on day 7 of culture ($P = 0.001$; Table 1). DNA concentrations increased from day 3 to day 5 and then decreased on day 7 of culture. In this study we have isolated and cultured the endometrial stromal cells from buffalo

Table 1. Growth pattern of endometrial stromal cells in culture

Day of culture	Protein (µg/25 µl)	DNA (µg/25 µl)
Day 3	6.97±1.59 ^a	11.19±0.51 ^a
Day 5	13.8±0.98 ^b	18.41±2.56 ^b
Day 7	30.55±1.77 ^c	13.5±0.54 ^{bc}
Significance	F = 50.346; P < 0.001	F = 7.762; P = 0.009

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Non-identical superscripts are significant at 5% level of significance.



Fig. 1. Endometrial stromal cells 36 h after plating. $\times 10$.

uterus. To the best of our knowledge and belief this is the first report of isolation and characterization of endometrial stromal cells. Separation and culture of stromal cells from endometrium were reported for rat (McCormack and Glasser 1980), rabbit (Fortier *et al.* 1987, Wang *et al.* 2009), pig (Wang *et al.* 2000) and cattle (Fortier *et al.* 1988) but not in buffalo. In our study, we used a slight modification of the method previously described by Yamauchi *et al.* (2001). Treatment of stromal cells with trypsin-EDTA eliminated the contaminating epithelial cells. Stromal cells are readily distinguishable from epithelial cells. This coupled with stromal cell inclination to remain unattached to plastic surface when subjected briefly to trypsin-EDTA, formed the basis of our separation technique. Endometrial stromal cells were flat and spindle shaped which are in agreement with earlier report in cattle (Fortier *et al.* 1988), sheep (Cherny and Findlay 1990) and pig (Zhang *et al.* 1991). The pattern of protein and DNA content in buffalo endometrial stromal cell culture agrees with earlier observations in bovines (Fortier *et al.* 1988) in terms of increase in protein and DNA concentration with the time in culture. The characterization of these cells using immune-histochemistry, electron microscopy or antibody labeling is not available in this species. Moreover, the involvement of stromal cells of buffalo endometrium with the luteotropic process during maternal recognition of pregnancy needs to be characterized. In conclusion, we have developed isolation and culture system for buffalo endometrial stromal cells that will provide an *in vitro* model for studying the interactions between epithelial and stromal cells.

SUMMARY

Isolation and characterization of endometrial stromal cells were carried out in order to investigate the role of stromal cells in implantation and pregnancy in buffalo. The stromal cells were found to attach within 36–48 h after plating and



Fig. 2. Endometrial stromal cells on day 7 of culture at the stage of confluence. $\times 10$.

exhibited fibroblastic shapes as well as contact inhibition at the stage of confluence. Protein concentrations have been found to increase progressively with the time in culture. DNA concentrations increased from day 3 to day 5 and then decreased to day 7 of culture. This is the first isolation, culture and characterization of endometrial stromal cells in buffalo.

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