The present study was carried out to characterize the morphological variations of bubaline oviductal epithelial cells derived during various stages of the oestrous cycle and maintained as short term cultures. The oviducts were collected from slaughtered sexually mature female buffaloes and divided into three groups based on the stage of the oestrous cycle, viz., early luteal phase (EP), mid luteal phase (MP) and late luteal phase (LP). A total of 18 trials, with six from each group, were conducted. The oviductal epithelial cells (OECs) were extruded and cultured for seven days. Morphological characterization revealed that OECs appeared as sheets of cells on day 0, changed into aggregates of worm-like or globular structures by day 1-2, revealed cell vacuolation and initiation of attachment by day 2-3 and reached sub-confluency by day 6. It was found that on day 1, the percentage of motile cells was significantly greater in the EP and LP groups (65.0 ± 10.5 and 63.3 ± 10.3 respectively) of OEC culture when compared to the MP group (48.3 ± 7.5%). On day 6, still significantly greater numbers of actively rotating motile cells were found in the EP (28.3 ± 9.8%) and LP (20.0 ± 6.3%) groups, while only 6.7 ± 8.2 percent of motile cells were observed in the culture of the MP group. Early initiation of attachment (day 1.5 ± 0.6) of a greater number of cells was noticed in the MP group when compared to the other two groups (day 2.3 ± 0.8 and day 2.0 ± 0.6 respectively). In the MP, the attached cells reached 70-80 percent confluency by day 6 of the culture, while only 30-40 percent confluency was observed in the EP and LP groups. It was thus concluded that oviductal cells isolated from the early luteal stages with increased ciliary activity and motile cells might be more appropriate for embryo co-culture experiments.

Keywords: Bubalus bubalis, oviductal epithelial cell culture, morphology, stage of oestrous cycle

INTRODUCTION

The oviduct plays a pivotal role in mammalian reproduction, providing an optimal environment for oocyte maturation, sperm capacitation, fertilization, and transport of gametes and embryos (Hunter, 2003). The oviduct epithelium consists mainly of two different cell types, viz., ciliated and non-ciliated (secretory) cells, and their relative proportions and morphology change markedly during the oestrous cycle (Leese et al., 2001). Cultured oviduct epithelial cells have been employed to overcome the developmental block at the 8-16 cell stage embryos generated in-vitro in ruminants (Galli et al., 2003). Undoubtedly, there is a practical need to increase knowledge about the
first environment to which embryos are exposed to improve IVF success rates and to ensure the normality of the embryos created. So, the present study was carried out to place on record the characteristics of the bubaline oviductal epithelial cells derived during various stages of the oestrous cycle and developmental pattern as short term cultures.

**MATERIALS AND METHODS**

The genital tracts were collected from sexually mature female buffaloes (5-8 years old) slaughtered at Corporation Abattoir, Chennai and transferred to the laboratory in a cool container (4°C) within 30-45 minutes for analysis. The reproductive organs were examined for normality and divided into three groups based on the stage of the oestrous cycle, viz., early luteal phase (EP), mid luteal phase (MP) and late luteal phase (LP), which was determined by the corpus luteum (CL) and follicular characteristics as described by Ireland et al. (1979). A total of 18 trials, with six from each group, were conducted.

**Preparation of buffalo oviductal epithelial cell culture**

The oviducts, ipsilateral to the ovary with CL, were excised from the reproductive tract, trimmed of excess connective tissue, ligated at the infundibular end and at the utero-tubal junction and washed twice in warm (37-39°C) sterile 0.9% saline. Oviducts were dipped in ethanol and washed with phosphate buffered saline before removing the ligature in a laminar flow hood. The oviductal epithelial cells (OECs) were extruded from the lumen of the oviduct by the stripping motion of a clean microscope slide over the exterior surface of the oviduct (Way, 2006). The extruded OECs from the lamina propria, which appeared as a yellowish paste, were collected in culture medium TCM-199 (Invitrogen) supplemented with 10 percent foetal bovine serum and 0.25 mg/ml gentamicin (Sigma). The cell suspension was pipetted 10-15 times with a 1000 μl filter tip, and cells were dissociated. After three steps of washing, each followed by 25-min sedimentation in culture medium in the water bath, cellular suspensions were dispensed into 25 cm² flasks (Falcon, Oxnard, USA) and cultured at 38.5°C in a humidity-saturated atmosphere of 5% CO₂ in air (day 0). The primary cultures were maintained for a short term of seven days (day 0-6) till they finally settled as a monolayer.

**Characterization of isolated cells and morphology of cultured OECs**

Morphological characterization of OECs derived from the three groups of oviducts was carried out every day (day 0-6) during the culture under a stereo zoom microscope. The day of initial attachment of OECs to the bottom of the flask in various groups was recorded. The proportion of motile cells on day 1 and day 6 of the culture was determined by arriving at the average percentage of motile cells observed under various fields of the culture. The proportion of confluency attained by the attached cells on Day 6 of the culture were analysed grossly. Data on the day of attachment and the proportion of motile cells between three groups were statistically analysed (Snedecor and Cochran, 1994).

**RESULTS AND DISCUSSION**

Morphological variations in OECs maintained as short term culture was represented
In general, freshly (day 0) isolated bubaline OECs appeared as sheets of cells with vibratory movements due to the presence of cilia on the periphery of epithelial cells. The mechanically obtained flat cell sheets changed into aggregates of worm-like or globular structures during the day 1-2 of culture and this morphology was maintained throughout culture. Cell aggregates with numerous cilia at the periphery remained in rapid and constant motion in the culture medium due to vigorous ciliary beating, but some cells without cilia were found to initiate their attachment process to the bottom of the flasks even as early as day 1-3 of the culture. Cell vacuolation was often detected by day 2-3, and ciliary beating was not found in such vacuolated cells (Hishinuma et al., 1989). On day 4 of the culture, the attached cells spread out extensively. The proportion of cells with ciliary activity decreased on day 5 of the culture. After attachment to the surface, the cilia could not be observed. The primary cultures reached sub-confluency by day 6. These observations were in concurrence with the findings of Rosselli et al. (1994) and Rottmayer et al. (2006).

The ciliated cells were spherical with cilia on their surface, and the cell mass showed the tendency to rotate in the medium. Proportions of ciliated and non-ciliated cell types were reported to change during the oestrous cycle (Yaniz et al., 2000). In the present study, as observed on day 1, it was found that the percentage of motile cells was significantly (P < 0.05) greater in the EP and LP groups (65.0 ± 10.5% and 63.3 ± 10.3% respectively) of OEC culture when compared to the MP group (48.3 ± 7.5%) as reported by Walter and Miller (1996) and Tienthai et al. (2009). On day 6, still significantly (P < 0.01) greater numbers of actively rotating motile cells were found in EP (28.3 ± 9.8%) and LP (20.0 ± 6.3%) groups while only 6.7 ± 8.2 percent of motile cells were observed in the culture of the MP group during the same period. A non-significantly increased proportion of motile cells was recorded in the EP group than the LP group. It was suggested that large numbers of ciliary cells in the oviduct were found during the follicular phase, but that their morphology altered by extensive atrophy and deciliation during the luteal phase due to cyclic changes affecting the hormonal environment (Suuroia et al., 2002).

The two cell populations of the oviduct epithelium showed different adhesion behaviour. Since the ciliated cells were in constant motion, the non-moving secretory cells seem to adhere easier and faster. This was best observed by the early initiation of attachment (day 1.5 ± 0.6) of a greater number of cells in the MP group, while in the EP and LP groups, the initiation of attachment of cells was noticed on day 2.3 ± 0.8 and day 2.0 ± 0.6 respectively. However, there were no significant differences between groups. This observation was in concurrence with Thibodeaux et al. (1992) who reported that a portion of oviductal cells often attached and formed monolayer (approximately 20%) during the 48-h incubation period. In the MP, the attached cells reached 70-80 percent confluency by day 6 of the culture, while only 30-40 percent confluency was observed in the EP and LP groups. The high percentage of confluency in the MP group could be attributed to the increased proportion of non-motile cells. Thibodeaux et al. (1992) opined that oviductal cells with increased ciliary activity, viability, and efficiency of attachment and growth, were suitable for in vitro embryo production studies. It could be concluded, based on the morphological characterization, that buffalo OECs derived from the early luteal stages of the oestrous cycle, during which highly motile cells were dominant,
Day of culture | Buffalo oviductal cells in culture | Remarks
--- | --- | ---
Day 0 |  ![Day 0](image) | Sheets of OECs with cilia in periphery
Day 1 |  ![Day 1](image) | Flat cell sheets changed into aggregates of worm-like or globular structures
Day 2 |  ![Day 2](image) | Vacuolation of cells
Day 3 |  ![Day 3](image) | 
Day 4 |  ![Day 4](image) | Attachment and spreading out of OECs
Day 5 |  ![Day 5](image) | 
Day 6 |  ![Day 6](image) | Confluent mono layer

Table 1. Figures of morphological changes in buffalo oviductal cells during short term culture.
might be more appropriate for embryo co-culture experiments.

REFERENCES


