Original Article

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MICROSCOPIC EXPLORATION INTO THE BEHAVIOR OF GIANT CELLS IN PLACENTOMES OF BUFFALO (*Bubalus bubalis*)

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ABSTRACT

The present study was conducted on placentomes of 31 pregnant buffaloes ranging from 38 to 243 days of gestation to explore the microscopic details of giant cells and their behavior in buffalo placentomes. Various histological and histochemical stains used in the study revealed its structural and chemical details. The study revealed that giant cells played a major role in transplacental transfer of nutrients and other metabolites required by the fetal and maternal tissues. High polysaccharide content and intense enzymatic reaction indicated high metabolic activity of the giant cells in the placenta. The migratory nature of giant cells observed in the present study revealed its role in transfer of metabolites. The cytoplasmic processes observed in the study indicated its fusion with the cryptal epithelium as a medium of transfer of metabolites and formation of multinucleated giant cells. Strong acid phosphates activity can be correlated with its erythrophagocytic nature as a medium of transfer of iron molecules to the developing fetus.

Keywords: *Bubalus bubalis*, buffaloes, placentomes, giant cells, histology, histochemistry

INTRODUCTION

The ruminant placenta comprises of unique structures termed as placentomes, which refers to a composite structure formed by the union of maternal caruncles with foetal cotyledons. In placentomes, numerous slender highly vascular and branched chorionic villi fit into the crypts of maternal caruncles vastly increasing the surface area for feto-maternal transport (Wooding, 2022). These villi are lined by a single layer of cells called as trophoblastic cells. Hradecky *et al.* (1987) described this villous-crypt interface as the main channel for trans-placental exchange of gases and metabolites. The presence of binucleate giant cells in the ruminant placenta is a characteristic feature and thought to be originated from the mononucleate trophoblastic cells by acytokinetic mitosis (Klisch *et al*., 1999). These binucleate giant cells have been reported as early as 16 to 17 days of pregnancy in

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ruminants (Aughey and Frye, 2001). The number of these cells increased with advancement of pregnancy. They are believed to play an important role in physiological exchange across placenta. They have been reported to migrate and fuse with the maternal epithelium forming multinucleated cells (Ranjan and Singh, 2013a). Lee *et al.* (1986) reported that the granule transfer seems to be the primary function of binucleate cell migration in the cow. Hence, the present study was undertaken to elucidate, microscopically, the behavior of these giant cells during gestation in buffaloes due to paucity of literature on histological aspect of placentomes in buffalo.

MATERIALS AND METHODS

The present study was conducted on placentomes of 31 pregnant buffaloes ranging from 38 to 243 days of gestation. These placentomes of early pregnancy were collected from the gravid uterine horn of non-descript buffaloes sacrificed at slaughterhouse and that of mid and late pregnancy during post-mortem examination. The gestational age was determined by measuring the curved crown rump length (CRL) of foetii using an inelastic thread as per formula given by Soliman (1975) in bovines. The samples were fixed in 10% neutral buffered formalin (10% NBF) and were processed by acetone-benzene schedule and these 5 to 6 µm thick paraffin sections were stained with Haematoxylin and eosin (H&E) for routine morphology, Periodic acid Schiff (PAS) for neutral-mucopolysaccharides, Alcian blue (pH 2.5) for acid-mucopolysaccharides and Prussian blue for ferric iron (Luna, 1968). The tissue samples were also processed for semi-thin sections (0.5 to 2.0 µm) and stained with 1% methylene blue.

For the demonstration of lipids, fresh unfixed tissue samples were subjected to cryostat sectioning at -20^{o}c and sections of 10 to 12 µm thickness were obtained and stained with Sudan black B (Chayen *et al*., 1969). For demonstration of enzymatic activities, the cryostat sections were incubated with the required incubating medium for Alkaline phosphatase (AKPase), Acid phosphatase (ACPase), Succinate dehydrogenase (SDH), Lactate dehydrogenase (LDH), and NADPH-diaphorase (Pearse *et al*., 1972). The positive and negative controls were used wherever possible.

RESULTS AND DISCUSSIONS

In the present study, three types of giant cells were observed in the placentomes of buffalo viz., uninucleate, binucleate and multinucleate giant cells (Figure 1). These giant cells were present in both the maternal (cryptal) and foetal (trophoblastic) epithelium. The binucleate giant cell was first evident at 4 cm CRL (47 days of gestation) within the developing primary crypt. The scattered uninucleate and binucleate giant cells in epithelium on both sides were less frequent upto 5.5 cm CRL (53 days of gestation). Their number increased from 9 cm CRL (69 days of gestation) in both the cryptal and trophoblastic epithelium. From 13 cm CRL (87 days of pregnancy) onwards, they showed more consistent appearance in both the maternal and foetal epithelium. Their number increased with the advancement of pregnancy. The binucleate giant cells were more in trophoblastic epithelium and multinucleate cells were more in cryptal epithelium. In late pregnancy, their number decreased on both sides, as compared to mid pregnancy. Lawn *et al.* (1969) also reported that at full term pregnancy in sheep and goat, the

binucleate cells were fewer in the chorionic villi while they were more numerous in the cryptal wall. The presence of these giant cells in the placenta had also been reported by various authors in different animal species at varying gestational age due to differences in the gestational length; but with relative similar time of occurance (Igwebuike, 2004 in ruminants and Schmidt, 2005 in buffalo). In the present study, the giant cells were also found isolated in the space between cryptal walls and trophoblastic epithelium and also within the core of villi at 40 cm CRL (164 days of gestation (Figure 2). These isolated giant cells might be the migrating giant cell from trophoblastic epithelium to cryptal epithelium.

In the present observations, the binucleate giant cells were strongly eosinophilic with darkly stained nuclei. The uninucleate giant cells had a less eosinophilic cytoplasm and lightly stained large nuclei with chromatin mass condensed at the periphery, as compared to binucleate giant cells. The giant cells were observed to be migrating and were present in the spaces between the cryptal and trophoblastic epithelium (Figure 3). Vacuolations in the cytoplasm of migrating giant cells were evident at 13 cm CRL (87 days) and afterwards the vacuolated cytoplasm in giant cells persisted throughout the pregnancy (Figure 4). Maximum migration of giant cells was noted at 31 cm CRL (143 days) and then afterwards the migration gradually decreased, and it was absent during late pregnancy. The cytoplasmic processes joining the cryptal and trophoblastic epithelium was well evident showing attachment between the cryptal multinucleate cell and the migrating trophoblastic giant cells (Figure 5) that disappeared at late gestation. Wooding (2022) had also reported that the binucleate cells (BNCs) migrate and form syncytium with the uterine epithelium to form trinucleate cells (TNCs) and play a major role in transfer of placental lactogens in the maternal circulation.

Extravasation of blood into the space between foetal arcade and maternal arches was observed from 49 cm CRL (184 days) onwards followed by clustering of migrating binucleated giant cells around extravasated erythrocytes in the arcade area near the villous base (Figure 6 and Figure 7). Extensive extravasation and subsequent clustering of giant cells around erythrocytes was further added on by intense acid phosphatase activity in the trophoblastic epithelium of arcade area that indicated the process of erythrophagocytosis (Figure 8). Finally, iron in the form of fine granules was observed in the trophoblastic epithelium of the villous base in the arcade area (Figure 9) during advanced pregnancy stage. All these events indicated active erythrophagocytosis by the giant cells to provide iron to the developing foetus. Thus the arcade area seems to constitute an important route for the transfer of iron from mother to foetus (Ranjan *et. al*., 2012). Myagkaya *et al.* (1984) had also demonstrated the acid phosphatase activity in erythrophagocytizing trophoblast in ovine placenta.

The neutral mucopolysaccharides content in the giant cells was intense especially in the arcade area (Figure 10). At 31 cm CRL (143 days) some of the migrating giant cells showed alcinophilic granules (Figure 11). Nandeshwar *et al.* (2006) also observed strong PAS reaction in binucleate cells of sheep and goat placentomes. Klisch and Leiser (2003) also reported the presence of a large amount of PAS-positive cytoplasmic granules in bovine binucleate giant cells which were identified as pregnancy-associated glycoproteins (PAGs). These secretory proteins of the BNCs suggested a functional role of this specific glycosylation

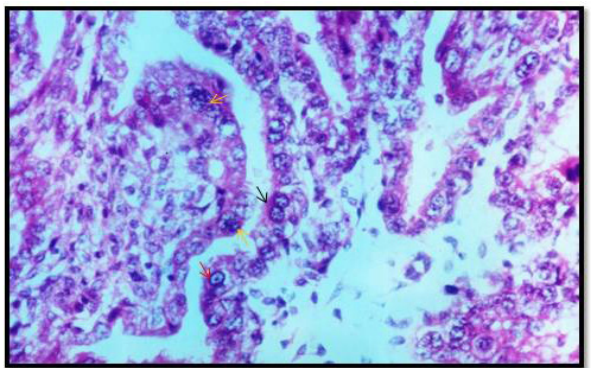

Figure 1. Microphotograph of buffalo placentome showing uninucleate (red aarow), binucleate (black arrow) and multinucleate (yellow arrow) giant cells. H&E 20x.

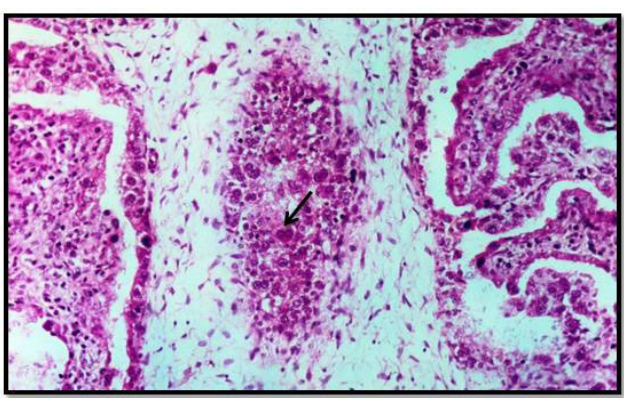

Figure 2. Microphotograph of buffalo placentome showing binucleate giant cells (black arrow) within the core of the villi. H&E 10x.

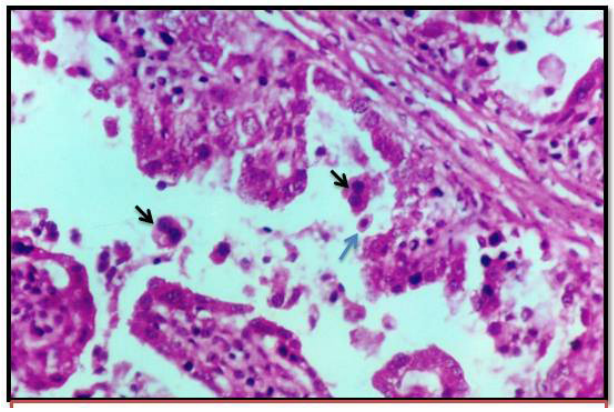

Figure 3. Microphotograph of buffalo placentome showing darkly eosinophilic binucleate giant cells (black arrow) and less eosinophilic uninucleate giant cells (blue arrow) migrating between the trophoblastic and cryptal epithelium. H&E 10x.

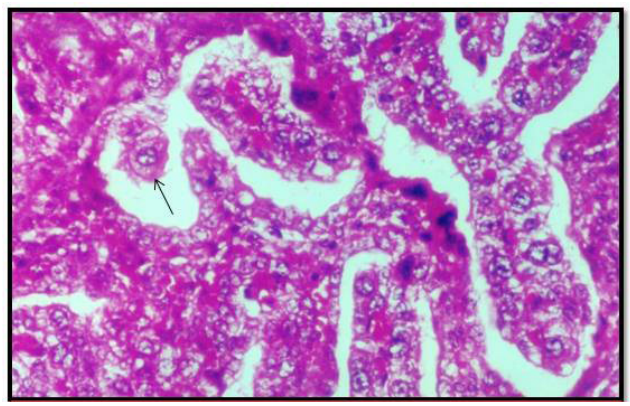

Figure 4. Microphotograph of buffalo placentome showing vacuolated cytoplasm of a migrating giant cell (black arrow). H&E 20x.

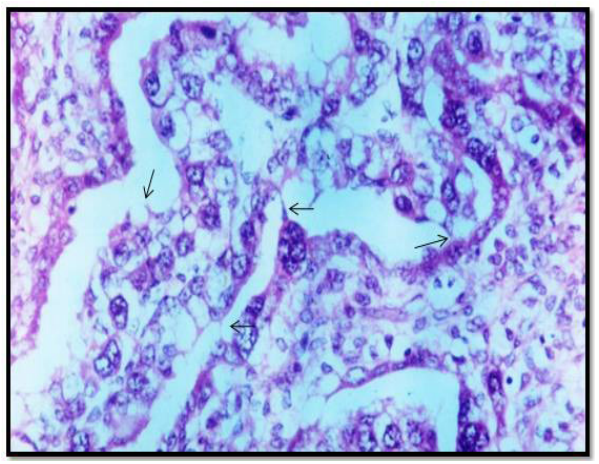

Figure 5. Microphotograph of buffalo placentome showing cytoplasmic processes of migrating giant cell and fusion to form multinucleated giant cell (black arrow). H&E 20x.

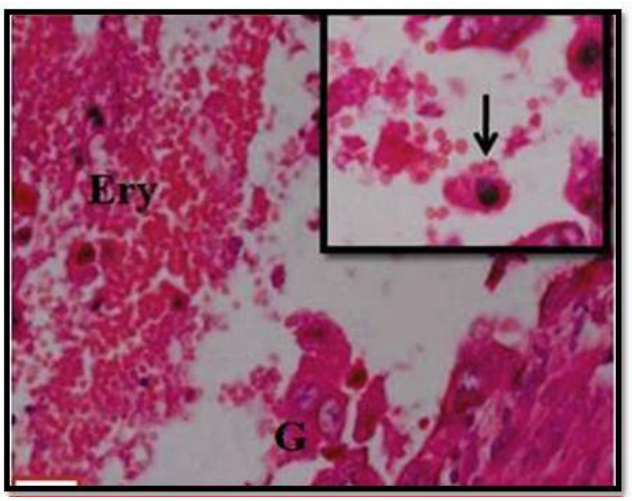

Figure 6. Microphotograph of buffalo placentome showing extravasation of erythrocytes and clustering of giant cells. H&E 20x.

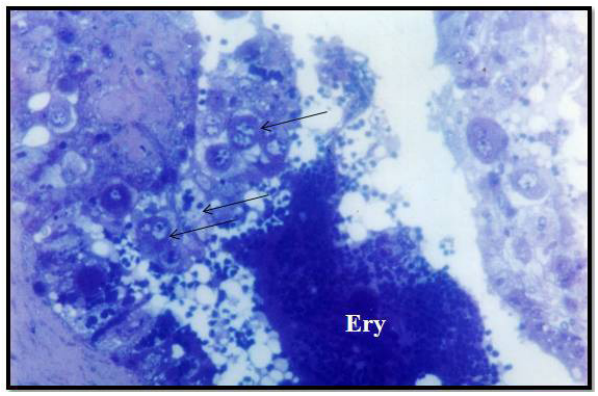

Figure 7. Microphotograph of buffalo placentome showing extravasation of erythrocytes in the arcade area and clustering of giant cells around erythrocytes. Methylene blue 40x.

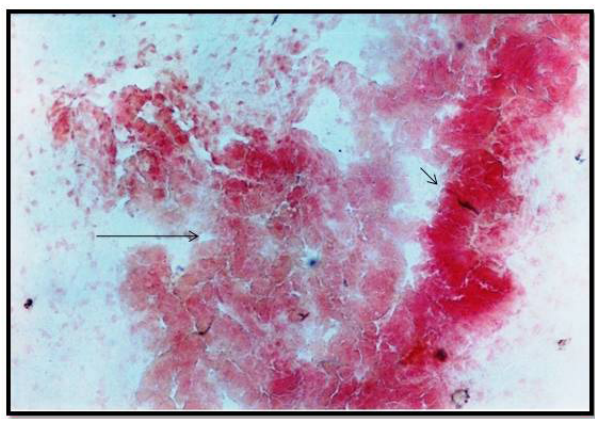

Figure 8. Microphotograph of buffalo placentome showing intense acid phosphatase activity in the trophoblastic ephithelium of the arcade area and extravasated area. Azodye method 10x.

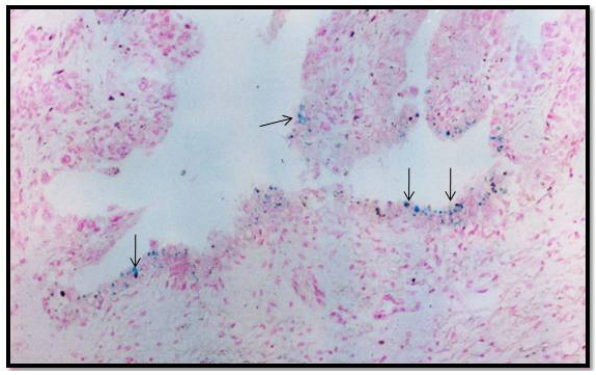

Figure 9. Microphotograph of buffalo placentome showing iron granules in the trophoblastic ephithelium of the arcade area and extravasated area. Prussian blue 20x.

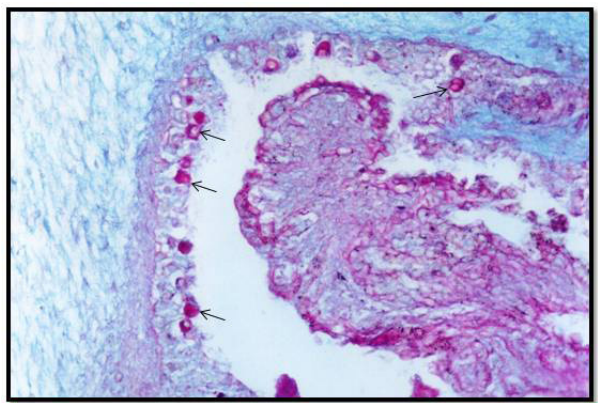

Figure 10. Microphotograph of buffalo placentome showing intense neutral polysaccharide content in giant cells especially in the arcade area. PAS-AB 10x

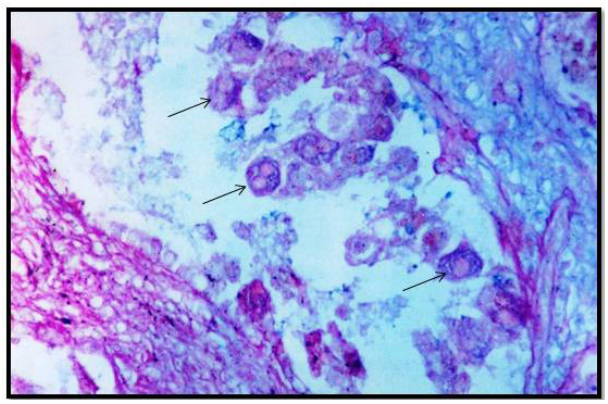

Figure 11. Microphotograph of buffalo placentome showing alcinophilic granules within the cytoplasm of migrating giant cells. PAS-AB 20x.

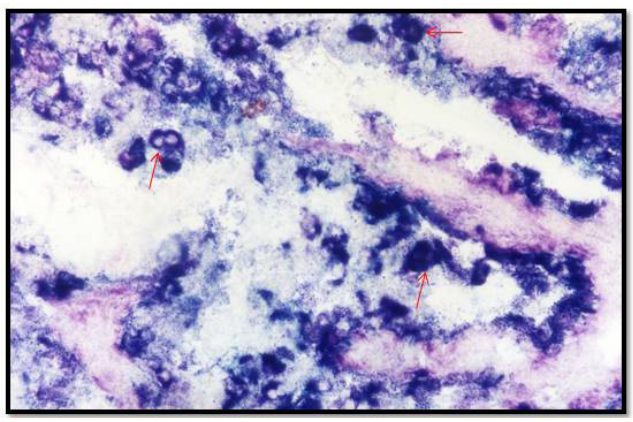

Figure 12. Microphotograph of buffalo placentome showing strong reaction for AKPase in the giant cells. Azodye method 10x.

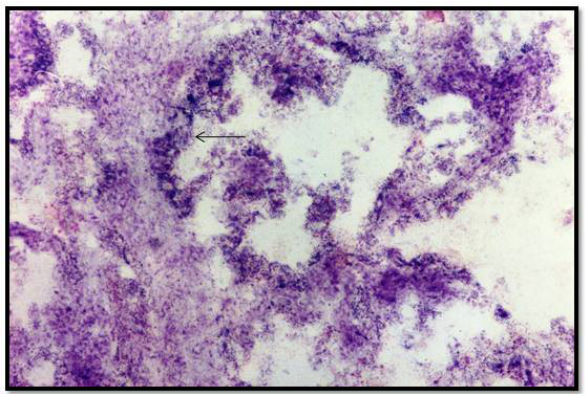

Figure 13. Microphotograph of buffalo placentome showing strong SDH activity in the giant cells. Nitro BT method 10x.

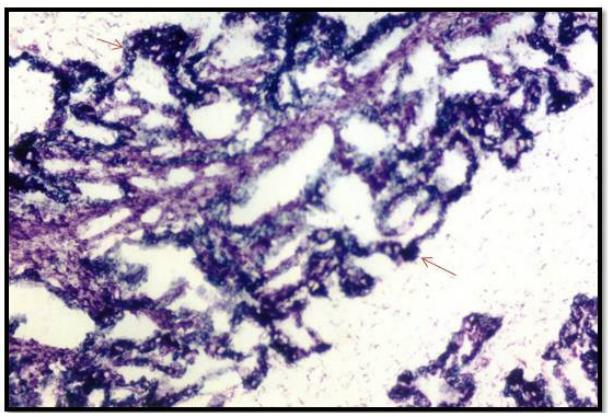

Figure 14. Microphotograph of buffalo placentome showing strong LDH activity in the giant cells. Nitro BT method 10x.

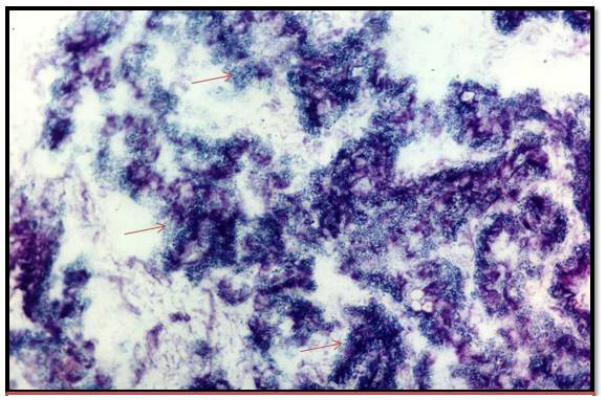

Figure 15. Microphotograph of buffalo placentome showing strong reaction of NADPH-diaphorase in the giant cells during the pregnancy Nitro BT method 10x.

pattern. In the present study, all cells containing mucopolysaccharides also showed high activity of dehydrogenases (Figure 13, 14 and 15), thus confirming that a high level of metabolism would be expected in placental trophoblast where considerable activity with respect to transport, degradation and synthesis of materials is taking place.

The trophoblastic giant cells showed intense positive reaction AKPase throughout the pregnancy (Figure 12) which indicated its role in process of absorption by active ion transport (Ranjan and Singh, 2013b). Leiser and Wille (1975) had also correlated AKPase activity in the placenta with the transport of metabolites for histiogenic uterine milk production.

The giant cells exhibited moderate to strong activity of SDH throughout the pregnancy in the present study (Figure 13) whereas, intense activity of LDH was observed in the giant cells throughout the period of gestation (Figure 14). High SDH and LDH activity in the giant cells indicated high metabolic activity. The increase in activity of this enzyme with the advancing gestation indicated increase in placental transport of nutrients to the foetus, as there is marked increase in weight in these species in the later part of pregnancy (Christie, 1968). Very strong reaction of NADPH-diaphorase was noted in the giant cells during the pregnancy (Figure 15). According to Shrader and Zeman (1972), NAD dependant enzyme is found in cells in which glycolytic pathway is active. It catalyse the formation of lactate in anaerobic glycolysis and pyruvate in aerobic respiration. NADPH-diaphorase activity reflects the metabolic activity of the cell.

CONCLUSION

The present study revealed that the giant cells in placentomes of buffalo play a major role in transport across the placental barriers as they are migratory in nature. The histological findings suggested that the presence of vacuolations and granules within the cytoplasm with intense enzymatic activity represented its high metabolic activity and role in placental transport of nutrients

and other metabolites. The strong acid phosphatase activity at the villous base observed in the study indicates its erythrophagocytic nature and its role in transfer of iron from mother to fetus especially during mid and advanced pregnancy. The cytoplasmic processes observed in the study indicated its fusion with the cryptal epithelium as a medium of transfer of its metabolites and formation of multinucleated giant cells. The abundant number of binucleated giant cells during early and mid-pregnancy can be correlated with its high functional activity required during that phase of pregnancy, while decrease in number during late pregnancy can be correlated with the preparation of fetal membrane detachment during parturition.

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