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UDC 636.3.09:611.013.8:611.018:611.068 **Original Scientific Article**

MORPHOLOGICAL ADAPTATIONS FOR HISTOTROPHIC NUTRITION IN THE PLACENTA OF WEST AFRICAN DWARF GOATS

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Summary: Gravid uteri harvested from 11 pregnant West African Dwarf goats were used to study morphological adaptations for secretion and absorption of histotroph in interplacentomal areas of the goat placenta. The results showed numerous glandular acini in the richly vascularized sub-epithelial connective tissue of the endometrium. Secretory cells of the glandular acini exhibited cytoplasmic organelles for synthesis and packaging of secretory products, as well as many membrane-bound secretory vesicles. At the foeto-maternal interface, microvillar interdigitations occurred between uterine epithelial cells and foetal trophoblasts. In addition, areolae were observed near the openings of uterine glands onto the endometrial surface. Trophoblast papillae, which are evaginations of the foetal chorioallantoic membrane, extended into the areolar cavity and lumina of uterine gland openings. The areolae are specialized sites for storage and absorption of uterine gland secretions, while trophoblast papillae may correspond to areas for substantial absorption of uterine gland secretions by foetal trophoblasts. Areolae and trophoblast papillae persisted up to day 90 of gestation in the West African Dwarf goat. This suggests that uterine gland secretions are required by the conceptus even after implantation and establishment of haemotrophic nutrition in the goat. Thus, this study has provided morphological evidence for substantial production and transfer of uterine gland secretions from the dam to the foetus in interplacentomal areas of the placenta of West African Dwarf goats.

Key words: uterine gland; foetal trophoblast papillae; areolae; interplacentomal area; placenta

Introduction

Ruminant placenta is classified as cotyledonary on the basis of its gross anatomical features. It exhibits discrete areas of attachment, the placentomes, which are separated by interplacentomal areas (1, 2). Placentomes are formed by interdigitation of long, profusely branched cotyledonary villi of the chorioallantois with deep caruncular crypts of the endometrium (3). Whereas placentomes are sites for haemotrophic exchange of nutrients and metabolites between the foetus and the dam, interplacentomal areas of the endometrium have been shown to contain large numbers of branched coiled uterine glands (4, 5). Endometrial glands synthesize and secrete or transport a variety of enzymes, growth factors, cytokines, lymphokines, hormones, transport proteins and other substances, collectively referred to as histotroph (4).

Studies conducted on uterine gland knockout ewes strongly suggest that uterine glands and their secretions are essential for peri-implantation

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conceptus growth and survival (6, 7, 8, 9). Embryo transfer experiments in uterine gland knockout ewes revealed that endometrial gland secretions begin to impact conceptus growth and development on day 11 of pregnancy (10). Available evidence supports the idea that secretions of endometrial glands are the primary regulators of conceptus survival, development, production of pregnancy recognition signals, implantation and placentation (5, 11, 12, 13). Indeed, uterine secretions contain proteins such as osteopontin, an acidic component of the extracellular matrix, which binds to integrin receptors expressed on endometrial luminal epithelium and conceptus trophoblasts, to stimulate changes in morphology of the trophoblasts and mediate adhesion between uterine luminal epithelium and foetal trophoblastic epithelium (14, 15).

Endometrial gland hyperplasia occurs between day 15 and 50 of gestation in ewes, followed by hypertrophy to increase surface area that allows for maximal production of their secretions after gestation day 60 (16). This suggests that foetal requirement for uterine gland secretions may not be restricted to the peri-implantation period in sheep. The objective of the present study is to investigate morphological adaptations that may enhance the secretion and absorption of histotroph in interplacentomal areas of the placenta of West African Dwarf goats, using light and transmission electron microscopic techniques.

Materials and methods

Animals

All procedures involving animals were conducted according to the guidelines for the protection of animal welfare at the University of Nigeria Nsukka.

The 11 female West African Dwarf goats used for this study were purchased from local markets in Nsukka Local Government Area, Enugu State, Nigeria. The animals were housed in goat-pens at the Animal House Unit of the Faculty of Veterinary Medicine, University of Nigeria Nsukka. Confirmation of pregnancy was by ultrasonography carried out at the Veterinary Teaching Hospital, University of Nigeria Nsukka. Stages of pregnancy were determined by recording the mating date and monitoring any return to oestrus. When does failed to return to service, the first day after the last mating was taken as the first day post coitum (dpc). Subsequent stages of pregnancy were determined from that date. The stages of pregnancy studied were 20, 45, 50, 75, 85, 90, 100, 110, 115, 130 and 140 days of gestation. Gravid uteri were harvested from the pregnant goats at slaughter immediately after exsanguination.

Histological preparations

Each gravid uterus was incised and interplacentomal areas of the placenta were carefully cut free and fixed by immersion in 10% neutral-buffered formalin. The samples were dehydrated in increasing concentrations of ethanol, cleared in xylene and embedded in paraffin wax. 5-6 μm thick sections were cut and stained with haematoxylin and eosin (H&E) for light microscopy. Photomicrographs were captured using a Moticam Images Plus 2.0 digital camera (Motic China Group Ltd.) attached to a Leica binocular microscope.

Transmission electron microscopy

Samples of interplacentomal tissues for transmission electron microscopy were fixed by immersion in modified Karnovsky's mixture containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.4. The samples were further post-fixed in 1% osmium tetraoxide in Millionig's buffer. Dehydration of the tissue samples was accomplished in increasing concentrations of ethanol and propylene oxide. Thereafter, the samples were embedded in epoxy resin and cured overnight in an embedding oven at 65°C. Semithin sections (1 μm thick) were cut and stained with toluidine blue for light microscopy. Ultrathin sections (50-90 nm thick) were cut and stained with Reynold's lead citrate and saturated aqeous uranyl acetate. These were examined under the Philips CM 10 transmission electron microscope. Images were captured using an Olympus MegaView III digital camera (Olympus Corporation Japan) attached to the transmission electron microscope.

Results

Light microscopic features

Uterine epithelial lining of interplacentomal areas of the goat placenta consisted predominantly of mononucleated columnar epithelial cells, whose apical surfaces made contact with trophoblastic epithelium of the foetal chorioallantois at the foetomaternal interface. There was simple apposition between the uterine epithelium and the foetal trophoblastic epithelium (Figure 1). The richly vascularized sub-epithelial connective tissue of the uterine wall contained many glandular acini (Figures 1, 2). Each glandular acinus was made up of columnar secretory cells that rested on a basement membrane (Figures 2, 3). Blood capillaries abut and indent the basement membrane of the glandular epithelium (Figure 2). Cytoplasm of the secretory cell stained predominantly basophilic, and the round or oval nucleus was located towards the base of the cell, while secretory products accumulated in the apical cytoplasm (Figure 3). Secretory cells appeared to release their products into the lumen of the acinus by apocrine method of elaboration (Figure 4).

Uterine glands opened onto the surface of the endometrium. The apposed foetal trophoblastic epithelium draped over uterine gland openings to enclose spaces known as areolae at the foetomaternal interface (Figure 5). Trophoblast papillae were observed as evaginations of the foetal chorioallantois that extended into the areolar cavity and lumina of uterine gland openings. They were present at day 90 of gestation (Figure 6). The external surface of the trophoblast papilla was lined by trophoblasts, while its core consisted of foetal connective tissue.

Transmission electron microscopic features

Interplacentomal uterine epithelium was composed of tall columnar cells that rested on a well-defined basal lamina and extended to the foetomaternal junction (Figure 7). Each cell possessed a single oval nucleus, and its cytoplasm showed abundant mitochondria and many cytoplasmic vacuoles (Figure 8). Adjoining uterine epithelial cells were bound together by apicolateral tight junctions, while the apical plasmalemma of these cells was modified into numerous microvilli for interdigitation with similar processes of the foetal trophoblastic epithelium (Figure 8).

Uterine glands of the goat placenta were made up of tall columnar or cuboidal secretory cells (Figure 9). Each secretory cell possessed a basally located oval nucleus that showed slight indentation. The nucleolus was prominent, and the outer membrane of the nuclear envelop was studded with polysomes. Major characteristics of the cytoplasm of the secretory cell were predominance of granular endoplasmic reticulum cisternae and accumulation of secretory vesicles in the apical cytoplasm of the cell (Figure 9).

Discussion

Endometrial glands occur in interplacentomal areas of the placenta of West African Dwarf goats. This is similar to the reports of previous studies in other ruminant species (4, 5). Ultrastructural features of the glandular epithelium in the goat suggest that the secretory cells are actively engaged in protein synthesis. The abundant granular endoplasmic reticulum may be responsible for the production of numerous membrane-bound secretory vesicles present in the cytoplasm of the cell. Moreover, variation in the shape or height of the secretory cells from tall columnar to low cuboidal forms may be related to the mode of elaboration of the secretory products by apocrine method.

It is obvious from our study that secretions of endometrial glands are made available to the conceptus through openings of uterine glands onto the endometrial surface. A previous study in sheep revealed that during the pre-implantation period, from day 4 to 15 of gestation, conceptuses are free-floating in the uterine lumen where they are bathed in and are thought to be supported by uterine gland secretions (17). In addition to providing nutrition for the conceptus, uterine gland secretions contain growth factors and cytokines that promote cell division, proliferation, morphogenesis and differentiation (18). It has been reported that uterine gland knockout ewes are unable to support pregnancy up to Day 25 of gestation, because the uterine gland knockout phenotype, characterized by extreme reduction in or absence of endometrial glands constitutes a uterine lesion that compromises peri-implantation conceptus growth and survival (6, 7, 10).

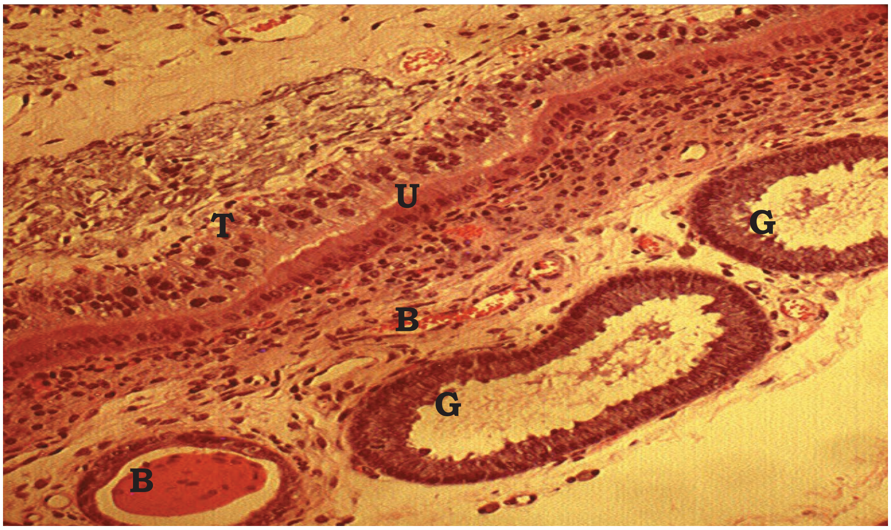

Figure 1: Interplacentomal area of the goat placenta showing simple apposition between foetal trophoblastic epithelium (T) and uterine epithelium (U). The uterine wall exhibited endometrial glands (G) and their associated blood vessel (B). H&E stain x100

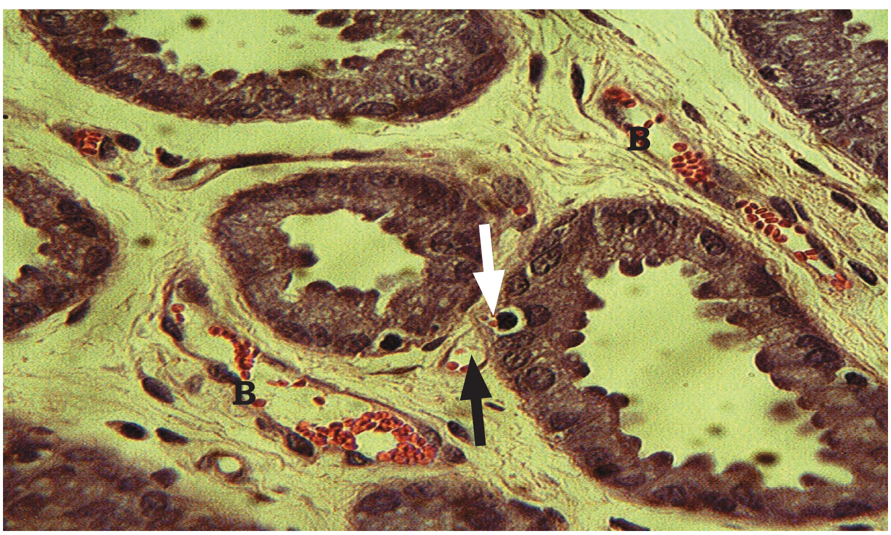

Figure 2: Photomicrograph showing blood vessels (B) associated with the endometrial glands. The blood capillary may abut (black arrow) or indent (white arrow) the basement membrane of the glandular acinus (arrows). H&E stain x400

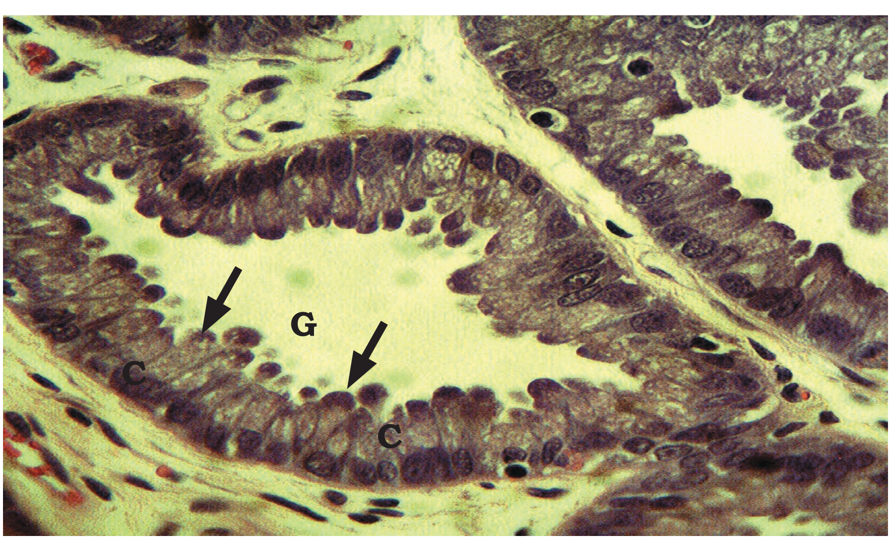

Figure 3: Uterine glandular acini (G) at day 85 of gestation showing columnar secretory cells. The secretory products accumulate in the apical cytoplasm (arrows) of the cells. H&E stain x400

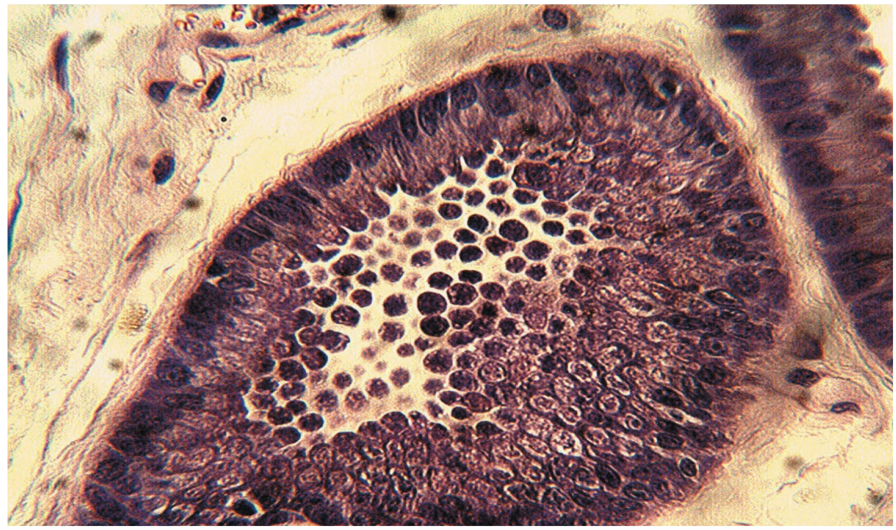

Figure 4: Uterine glandular acinus at day 140 of gestation. The cells elaborate their secretory products by apocrine method. H&E stain x400

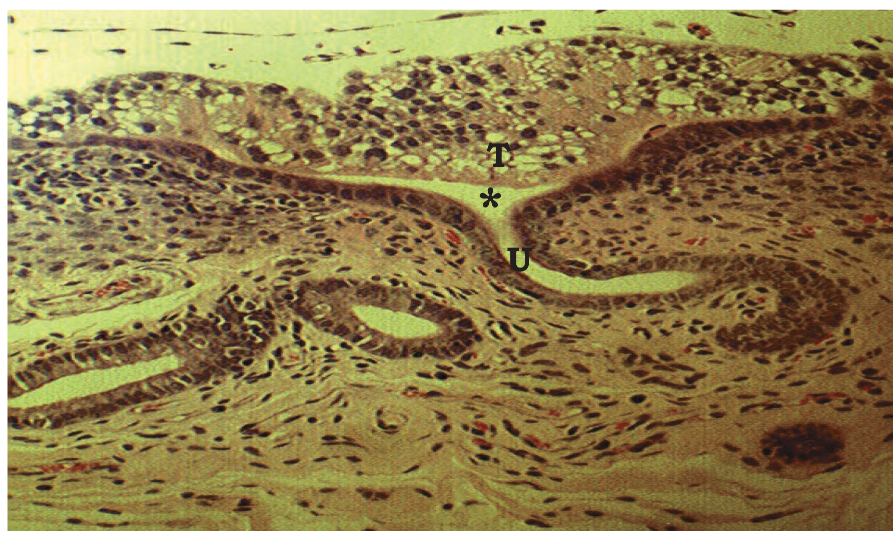

Figure 5: Photomicrograph of the interplacentomal area of a goat placenta at day 20 of gestation showing that the trophoblastic epithelium (T) drapes over the opening of uterine glands (U) resulting in the areola (asterisk). H&E stain x100

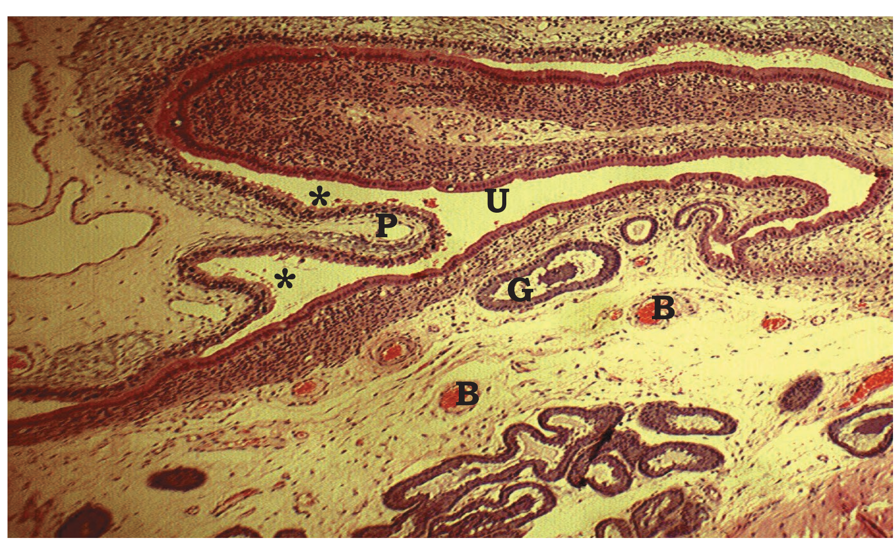

Figure 6: Photomicrograph of the interplacentomal area of a goat placenta at day 90 of gestation. A trophoblast papilla (P) extends into the areolar cavity (asterisk) and into the opening of uterine glands (U). Note glandular acini (G) and blood vessels (B). H&E stain x40

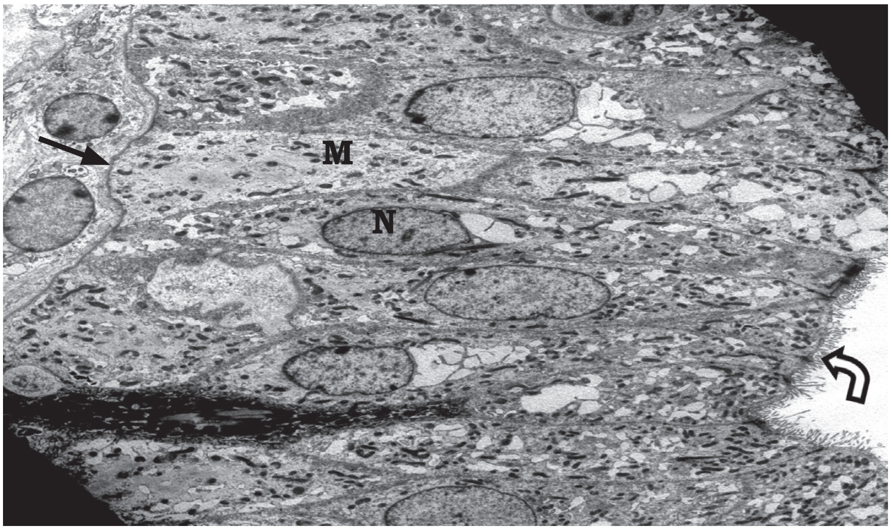

Figure 7: Electron micrograph of the interplacentomal uterine epithelium at day 110 of gestation showing tall columnar cells (M) that rest on a basal lamina (arrow). The apical cell membrane is modified into microvilli (curved arrow). Note the nucleus (N). TEM x1450

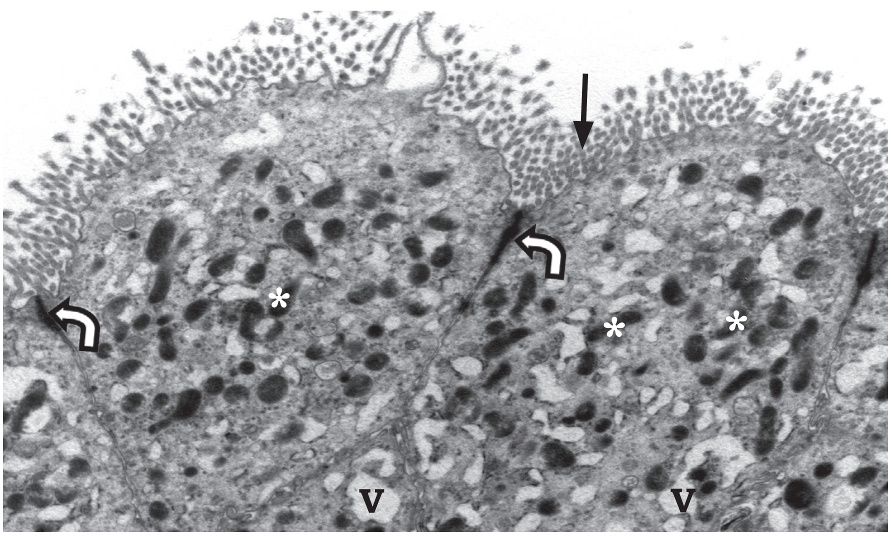

Figure 8: Apicolateral tight junctions (curved arrows) occur between adjoining uterine epithelial cells. Note numerous mitochondria (asterisks) and vacuoles (v) in their cytoplasm, as well as microvillar modification of the apical cell membrane (black arrow). TEM x5800

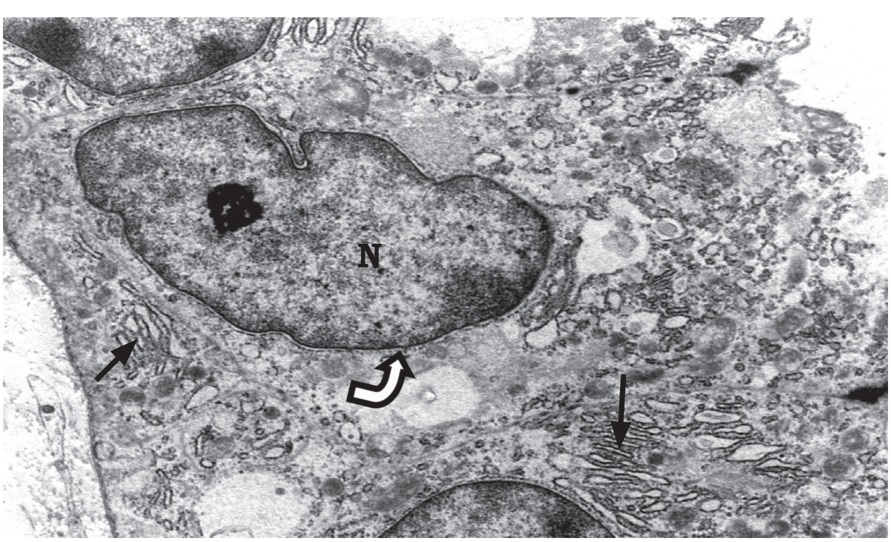

Figure 9: Micrograph of the uterine glandular secretory cells showing oval nucleus (N) with a slight indentation and a prominent nucleolus. The cytoplasm contains many cisternae of granular endoplasmic reticulum (arrows). The outer membrane of the nuclear envelop (curved arrow) is studded with ribosomes TEM x5800

One of the morphological features observed in this study is association of several blood vessels with endometrial glandular acini. This may facilitate exposure of the glandular epithelium to the influence of hormones and other substances present in maternal blood stream. This suggestion is supported by reports that the ruminant endometrium is exposed sequentially to oestrogen, progesterone, interferon-tau, placental lactogen and placental growth hormone during pregnancy (19, 20). These hormones constitute a servomechanism that activates and maintains remodeling of the endometrium, as well as endometrial gland morphogenesis and differentiated secretory function. In particular, placental lactogen, which is produced by trophoblast binucleate cells of ruminant conceptuses (21, 22), was detected in ovine maternal serum from day 50 of gestation, with peak levels occurring between 120 and 130 days of pregnancy (21, 23). Temporal changes in conceptus production of placental lactogen are correlated with endometrial gland morphogenesis and increased production of uterine milk protein and osteopontin by the glandular epithelium (14, 16, 24, 25).

Areolae occurred near the openings of uterine glands in the placenta of West African Dwarf goats during the peri-implantation period and up to the second trimester of pregnancy. Placental areolae have been demonstrated in sheep and pigs as specialized sites for storage and absorption of uterine gland secretions by the conceptus (1, 13). In addition, absorption of uterine gland secretions by the conceptus may be enhanced by the microvillar interdigitations between uterine and trophoblastic epithelial layers. The microvillar processes make for increase in surface area between maternal and foetal components of the placenta. Furthermore, trophoblast papillae may correspond to areas for substantial absorption of uterine gland secretions by the conceptus. Although previous studies showed that trophoblast papillae are temporary structures that disappeared by day 20 of pregnancy in sheep placenta (26) or occurred between day 14 and day 23 of gestation in goat placenta (27), our results indicate that trophoblast papillae are present in West African Dwarf goats at day 90 of gestation. The persistence of trophoblast papillae up to the second trimester of pregnancy in West African Dwarf goats may be proof that uterine gland secretions are required by the conceptus even after implantation and establishment of haemotrophic nutrition in the goat. It suggests that histotrophic nutrition complements haemotrophic nutrition and influences conceptus development, and growth of the foetus and placenta. Indeed, components of histotroph, the insulin-like growth factors (IGF-1 and IGF-2) have been shown to modulate foetal and placental growth (28, 29).

In conclusion, our study has provided clear morphological evidence for substantial production and transfer of uterine gland secretions from the dam to the conceptus in interplacentomal areas of the placenta of West African Dwarf goats. It supports the idea that conceptus requirement for histotroph remains critical even after implantation and establishment of haemotrophic nutrition in the goat.

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MORFOLOŠKE PRILAGODITVE HISTOTROFIČNE PREHRANE V PLACENTI ZAHODNOAFRIŠKIH PRITLIKAVIH KOZ

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Povzetek: Za raziskavo morfoloških prilagoditev za izločanje in vsrkavanje histotrofe na področjih med placentomi v placentah koz so bile uporabljene maternice 11 brejih zahodnoafriških pritlikavih koz. Rezultati so pokazali številne žlezne acinuse v subepitelijskih področjih endometrija, ki so bili bogato ožiljeni. Sekretorne celice žleznih acinusov so v citoplazmi celic vsebovale organele, potrebne za tvorbo in združevanje izločevalnih produktov, kakor tudi veliko mešičkov, povezanih z membranami. Na področju, kjer se stikata placenta matere in placenta zarodka/plodu, so bile opažene interdigitacije mikrovilov. Areole so bile opažene na področju prehoda materničnih žlez v področje endometrija. Papile trofoblasta, ki so invaginacije plodove horioalantoisne membrane, so se razširile v notranje področje areol in področja lumnov izvodil materničnih žlez. Areole so specializirana mesta za shranjevanje in vsrkavanje izločkov materničnih žlez, medtem ko papile trofoblastov lahko ustrezajo področjem močnega vsrkavanja izločkov materničnih žlez, ki jih vsrkavajo plodovi trofoblasti. Areole in papile trofoblastov so bile pri zahodnoafriških pritlikavih kozah prisotne od 90. dneva brejosti naprej. Ta podatek kaže, da so izločki materničnih žlez pomembni za vzdrževanje brejosti tudi po vsaditvi zarodka in vzpostavitvi hemotrofične prehrane pri kozah. Študija je priskrbela morfološke dokaze za močno produkcijo in prenos izločkov materničnih žlez od matere do zarodka na področjih med plancentomi v placenti zahodnoafriških pritlikavih koz.

Ključne besede: uterine žleze; papile fetalnega trofoblasta; areole; področja med plancetomi; placenta