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SOME MORPHOLOGICAL AND HISTOCHEMICAL STUDIES ON THE PLACENTA OF GOAT

BY

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This Thesis is Dedicated

with Appreciation

To

The Soul of my Sun

CONTENTS

INTRODUCTION

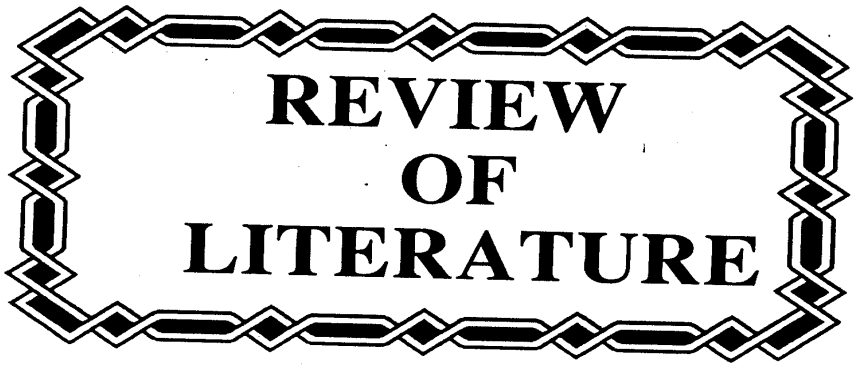
The placenta is an organ that develops during pregnancy. It is formed as a result of various degrees of interaction between the fetal and maternal tissues within the pregnant uterus. It consists of a fetal portion formed by the chorion and a maternal portion formed by the desidua basalis. The two portions are involved in physiological exchange of substances between the maternal and fetal circulation. Under normal conditions, the blood of the fetus and the blood of the mother neither mix nor come into direct contact with one another. They are always separated by what is termed the placental barrier *(Arey, 1974)*.

The placenta play an important role in the developing and growth of the fetus. Its primary function is to permit nutrient substances and oxygen dissolved in the mother's blood to diffuse through the placental barrier into the blood stream of the fetus. In addition, the placenta provides protection to the fetus and acts as an endocrine organ *(De Lahunta and Noden, 1985)*.

In ruminants, the placenta possesses highly vascularised, circumscribed and rounded thickening of lamina known as cotyledons, which act as points of attachment of the fetus with the uterus.

Reviewing the current literatures, despite of the extensive studies on the ruminant placenta, few were traced concerning the structure of the placenta of goat. For this reason and due to the

economic values of goat for meat and milk production as well as advantage in their fecundation, the present study was aimed to give some detailed informations on the histology, histochemistry and ultrastructure of the goat placenta at different stages of gestation period, with reference to the structure of the ripe placenta.


REVIEW
OF
LITERATURE

REVIEW OF LITERATURE

The goat placenta is syndesmochorial in type. In this type of placenta, the uterine epithelium disappears leaving the chorionic epithelium in contact with the endometreial connective tissue. Particularly all syndesmochorial placenta are cotyledonary or multiplex, a condition which is determined by the presence, from an early period and independently of pregnancy, of numerous endometrial caruncles which project as thickened knobs of the sub-epithelial tissue into the uterine lumen ***(Amoroso, 1952, 1961)***. The caruncles were found spread over the whole mucosa membrane including the tip of the uterine horn in goat ***(Lyngset, 1968)***. However, ***Nair and Raja (1973)*** stated that, the maternal caruncles in goat were spread over mucosa membrane except at the tip of the uterine horns. The distribution of the caruncles was reported to be regular in four rows on the placenta of single and twin pregnancy ***(lyngset, 1968; Nair and Raja, 1973)***. However, uneven distribution of the caruncles was observed by ***Lyngset (1968)*** in few cases especially in the left horn.

In early pregnancy, a great deal of the caruncles did not take part in placentation, this is more evident in the tip of the non-pregnant horn in single pregnancy. As pregnancy advanced, more caruncles were sharing in the placentation, however, even in advanced pregnancy, more caruncles were present than those actively participating in placentation ***(Lyngset, 1968 and Nair and Raja, 1973)***.

Concerning the shape of the maternal caruncles in doe (she-goat), ***Nair and Raja (1973)*** mentioned that the caruncles of the goat had a saucer-like shape and the cotyledons are fastened in the formed concavities which are either rounded or somewhat elongated in shape.

With regard to the number of the caruncles in the endometrium and cotyledons of the placenta in the goat, ***Martin (1904)*** reported that, the number of the caruncles in the uterus of the doe was 88-96, while ***Lyngset (1968)*** found that the uterus contain 120-125 caruncles.

With the conception, the caruncles form connection with localized villous proliferation of the chorion. The groups of chorionic villi are known as cotyledons, and together with the caruncles form the functional units that are often referred to as placentomes ***(Wimsatt (1950).*** He also reported that, in early stage of gestation, the placentomes become visible at 22 days of gestation which appears as minute rounded areas about one third millimeter in diameter. At day 26 of pregnancy, they were in the form of small circular thickenings. From day 28-32, they elongated to 0.5 millimeter; and at the 44^{th} day of gestation, most of the placentomes had a central depression, while in the mid-gestation, extended from the 7^{th} to 14^{th} week, the placentomes increased in diameter up to 1.5-2 mm as a result of stretching of the chorion. In the trimester (7 weeks before the parturition), the placentomes were about 3 mm in diameter.

Green and Winters (1945) stated that the placenta of ewe was developed 10 days after ovulation. However, ***Amoroso (1952)***, in the same

animal, found that, the first attack upon the maternal uterine mucosa occurs between the 17th and 18th day after service, where very loose attachment of membrane to caruncles. At about the 31st day, the villi appear on the surface of the chorionic sac resulting in closer attachment. At the 44th day, the fetal cotyledons are visibly arranged in rows and have convex free surfaces which fit into the concavities of the maternal cotyledons. The general features of the rip placenta occur at the 78th day, where by this stage there is a fair degree of adherence between maternal and fetal parts.

Wooding and Staples (1981) and Wooding et al. (1982), in ewe, found that at the day 13 of pregnancy, multicellular protrusions "papillae" from the trophoectodermal surface were protruded into the lumina of the uterine glands of the intercaruncular area close to developing embryo. By the day 16, they extent deeply into the glands. These papillae have not been observed at/or after the day 20 of gestation. They added that, these papillae were shown to be restricted to the embryonic region of conceptuses flushed out of the uterus and may play an important but transient role in anchoring the embryonic region of the conceptus against the uterine epithelium to allow the initiation of the cellular changes characteristic of the implantation; as reported also in cow by ***Guillomot and Guay (1982)***.

The histological structure of the placenta of the goat is nearly similar, even in ultrastructural level, to that of sheep as stated by ***Lawn et al. (1969)***.

Assheton (1906); Amoroso (1951, 1952 and 1961); Bjorkman (1965); Lawn et al. (1963, 1969); Daves and Wimsatt (1966) and Wooding et al. (1982) reported that the ovine placenta consists of chorionic villi fitting into maternal crypts separated from each other by septa. The chorionic villi consist of a mesenchymal cores covered with cuboidal trophoblastic cells and binucleated giant cells.

The formation of the crypts and cryptal epithelium was described in ewe by *Assheton (1906) and Amoroso (1951, 1952)* as a result of denudation or shedding of the uterine or caruncular epithelium. This was brought about at first by the agency of darkly staining binucleated cells of the trophoblast with the aid of phagocytic cells. The binucleated cells migrate through the uterine epithelium and come to be on the underling stroma, in their passage, they become enlarged and give rise to a layer of cells which seen to enter into very close relations with the cells of the stroma. At the 20^{th} day of pregnancy, the complete destruction of the uterine epithelium occur; it is mainly replaced by an irregular and interrupted layers of enlarged multinucleated trophoblastic cells. This denudation also occurs in the intercotyledonary area by contact of the foetal trophoblast with the mucosa of the uterine epithelium, then large polyhedral cells occur over the abraded surface just as they do over the placentomes. These cells are foetal and not maternal in origin, consequently, the placental membrane is of syndesmochorial rather than the epitheliochorial type in accordence to that established by *Grosser (1909)*. They added that, The denudation of the intercotyledonary uterine epithelium was more widespread in the ewe

and persists until midway through the fourth month of pregnancy, after which, the epithelial lining appears to be restored.

Boshier (1969) stated that, no evidence was obtained to support proposals that the trophoblastic binucleated giant cells in ewe have a major role in modifying or as a source of the cryptal epithelium. He added that the cryptal epithelium appears to be derived from the symplasmic plagues of maternal epithelium during the first 6 weeks of pregnancy.

Lawn et al. (1963) and Bjorkman (1965) found that in goat and sheep placenta there is a prominent microvillous interdigitation between the trophoblastic epithelium of the villus and the epithelium lining the crypts. This interlacking of foetal and maternal microvilli initiated series of changes in the uterine epithelium which transformed into partially syncytial crypt lining of the placentome. The microvilli occur at the basal as well as at the apical surface of the cryptal lining ***Lawn et al. (1969).***

The intercotyledonary area in the goat placenta was studied by ***Dent (1973)*** who reported that, a continuous epithelial layer present between the trophoblast and maternal blood vessels, this epithelium was partly cellular and partly syncytial in characters. He distinguished three types of syncytia : (a) flat syncytium, resemble columnar cells in ultrastructure, (b) swollen syncytium, which was vacuolated, and (c) degenerated syncytium, which condensed and appeared to be invaded and ingested by the trophoblastic processes from overlying trophoblast.

He also added that, within the uterine epithelium of pregnant goat, there is a continuous cycle of syncytial transformation, swelling and degeneration, the degenerated tissue was ingested by the trophoblastic cells and the epithelium was regenerated from its own basal cells.

Boshier and Holloway (1976, 1977) described the trophoblast as a cuboidal epithelium containing binucleated giant cells and separated from the mesenchym by well-defined basement membrane. The apical edge of this cell has been shown to be possess numerous microvilli which interdigitated with the microvilli of the maternal syncytium. They added that, in the last third of pregnancy, the cuboidal cells are mononucleate and have two forms, one form contains sparse elongated profile of rough endoplasmic reticulum, and the other contains dense polymorphic granules. These cuboidal cells may be correlated with secretion of steroid hormones, the production of ovine lactogen and the performance of normal non-endocrine placental activities.

According to the statement of Wimsatt (1951), the giant cells are commonly known as "diplokaryocytes" or binucleated cells which are present throughout gestation period and confined to the chorion in most species. On the other hand, Wimsatt (1951) and Lee et al. (1985) stated that, the distribution of these cells in sheep placenta are numerous at the tip of the villi, least numerous along the sides of the villi and moderately plentiful in the chorion between the bases of the villi and in the membranous chorion including the areolae. They added that the uterine epithelium of the placental crypt was lost early in gestation, but the giant cells from the villi attach themselves to the exposed connective tissue and give rise there to a primitive trophoblastic syncytium.

The binucleated cells are trophoblastic in origin, so they are specialized columnar trophoblastic cells and the characteristic duplication of their nucleus is accomplished by mitosis rather than by miosis, these cells, in certain parts of the placenta, are erythrophagocytic and probably assist in making iron available to the foetus ***(Amoroso, 1952; Davies and Wimsatt, 1966 and Wooding (1984).***

Wooding et al. (1981) and Wooding (1983, 1984) stated that, in ewe, the binucleated giant cells appeared at the day 14 post-coitum. By the 16^{th} day of pregnancy, 15-20 % of the trophoectodermal epithelium cells of the cow, sheep and goat placentomes were found to be binucleated cells; about 1/5 of these cells were migrated up to and cross the microvillar junction at all stages of pregnancy. This migration appears not only to transfer a characteristic granules of foetal binucleated cells to the maternal circulation, but also the formation of the placentomal syncytium bounding the maternal connective tissue in the goat and sheep. ***Boshier and Holloway (1977)*** described that, the binucleated giant cells of the sheep appear to be of two types, one concerned with glycogen storage and the other with synthesis of glycoprotein secretory substance. They added that, the binucleated cells are always enclosed by a thin continuous lamina of cuboidal cells cytoplasm which separated them completely from the maternal syncytium and the foetal connective tissue.

The placental barrier is that layer separating the maternal and foetal blood. In ovine placenta, it consists of maternal endothelium, maternal stroma, cryptal syncytium, trophoblast (comprising cuboidal

cells and binucleated giant cells), mesenchyme and foetal endothelium. In this placental barrier, the maternal syncytium is provided with faint basement membrane and the trophoblast rest on a thick basement membrane. The mesenchyme mainly by-passed by the occurrence of the intraepithelium capillaries and the maternal connective tissue was partly by-passed by direct contact between the maternal capillaries and cryptal syncytium *(Bjorkman, 1965, 1973).*

Davies and Wimsatt (1966) as well as Lawn et al. (1969) reported that, in the ovine placentomes, the barrier was reduced in thickness by the formation of intra-epithelial capillaries in the last month of pregnancy. These capillaries were intrude deeply into the epithelium of the villous, but preserve their independent endothelial basement membrane and truly extracellular, this occurs only in the placentome and not in the intercotyledonary area.

The placenta is the most reactive organ histochemically, some portions of it react positively with PAS technique; and the strongest positive reaction was observed where the embryonic and maternal tissues come into close contact *(Wislocki and Padykula, 1961 and Boshier, 1969).* In goat and sheep placenta, the PAS positive reaction (as indicator for carbohydrates) was demonstrated in the trophoblastic binucleated giant cells *(Wimsatt, 1951; Datta et al., 1979 and Kosaric et al., 1986),* in narrow amorphous layer between the trophoblast and the caruncular epithelium as well as in a line present between the uterine epithelium and stroma *(Boshier (1969),* in the maternal intercryptal columns *(Datta et al., 1979)* and in the luminal border and basement

membrane of the surface and glandular epithelium of the endometrium ***Bhattacharya and Saigal (1984).***

The acid phosphatase activity showed a moderate increase immediately before or during the implantation process, then the activity of the enzyme revealed a marked increase in the placenta during the early pregnancy in sheep ***(Hafez and White, 1968).***

Boshier (1969) found that a strong acid phosphatase activity was observed within fine granules in the apical regions of the uterine epithelial cells of pregnant ewe from the 14th to the 16th day of pregnancy. By the 16th day of pregnancy, this superficial response was accompanied by a low level of enzyme activity in the subepithelial stromal cells. During the end of the 3rd week of pregnancy, the acid phosphatase activity was still present in the caruncular epithelial area then declined during the 4th week and only chiefly demonstrated in stromal tissue beneath the degenerating maternal epithelium.

Zamiri (1980) stated that, the lowest activity of acid phosphatase was found in the uterine gland, luminal epithelium, stroma and circular myometrium of the sheep placenta, while the highest activity was observed in maternal caruncles. With the progress of the pregnancy, the acid phosphatase activity increased in the uterine glands, which showed a moderate acid phosphatase activity on day 80 of pregnancy.

Roy and Saigal (1987) reported that, in the sheep placenta, the acid phosphatase activity was very strong in maternal septa and cryptal

epithelium, it appears in form of fine granules evenly distributed at the perinuclear area, while moderate activity was seen in the endometrial glands and lamina epithelialis.

Hafez and White (1968) reported that the alkaline phosphatase activity was very low immediately before or during the implantation process in ewe, then increased gradually during the early pregnancy, reaching its maximum level at days 13 to 17 of pregnancy, then decreased sharply on days 18-19 and declined steadly until days 26 and 35.

Boshier (1969) found that the alkaline phosphatase activity during the early stage of pregnancy in sheep was present in both the luminal surface of the uterine epithelium and the sub-epithelial capillaries within the endometrial stroma, which maintained throughout the 3rd and 4th week of gestation and it was found at the junction of the trophoblast and maternal cryptal epithelium and was particularly apparent during the 4th week on the surface of the trophoblastic cells adjacent to the caruncle.

The activity of the alkaline phosphatase enzyme in the intercotyledonary endometrium of the sheep was mainly located in the cells of the surface and glandular epithelium, this activity showed a marked increase at the 8^{th} day of pregnancy, while a weak activity was detected in the subepithelial stromal cells during this time ***(Murdoch, 1970).***

Datta et al. (1979) mentioned that in the sheep placenta, the alkaline phosphatase enzyme was demonstrated primary in blood vessels of the surface region of maternal cotyledons, in the glandular epithelium and the giant cells. However, *Zamiri (1980)* stated that, the activity of the alkaline phosphatase was high in the luminal epithelium, uterine gland and caruncles and did not change during pregnancy.

Roy and Saigal (1987) reported that the fine granular intense alkaline phosphatase activity was found in the perinuclear area of the cells of the endometrium surface and glandular epithelium in early placentome of ewe, while the chorionic leave and its mesenchymal cells revealed a weak alkaline phosphatase activity, and the capillaries of the maternal septa, the cryptal cells and trophoblast had identical moderate to strong alkaline phosphatase activity.

In early pregnant ewe, the level of succinate dehydrogenase was low in the intercaruncular area of the endometrium *(Hafez and White, 1968)*, while in non pregnant sheep, the succinate dehydrogenase activity was higher in the caruncular epithelia and stroma, then in the myometrium, where only a moderate level of enzyme activity was demonstrated *(Zamiri and Blackshow, 1979)*.

Roy and Sagial (1987) reported that in early placentome of the sheep the succinate activity was strong in the uterine glandular and surface epithelia, also in cryptal cells. The maternal septa were moderately positive, while chorionic leave and mesenchymal cells of the

chorionic villi are weakly positive. A granular succinate dehydrogenase activity was observed in perimetrium, myometrium and endometrium stroma, it is weak to moderate. The fine granular perinuclear activity was evenly distributed in the glandular epithelium but occur intensely in the periphery of the trophoblasts and the uterine cryptal cells.

MATERIALS AND METHODS

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For the histomorphological studies, specimens were obtained from pregnant uteri of 80 apparently healthy Baladi goats at different gestation period, slaughtered at Cairo and Kafr El-Sheikh Abattoirs. The pregnant uteri were dissected and the ages of the embryos or foetuses were calculated from the CVR length according to the equation of ***Curson and Malan (1936)***. The CVR lengths of the embryos or foetuses were ranged from 1.5 cm - 32 cm (full term). Specimens from the placental tissue including the placentome and intercotyledonary area were taken from both the gravid and non gravid horns of the collected uteri shortly after the evisceration.

The specimens were excised into small pieces and fixed in 10% neutral buffered formalin for about 24-72 hours and in Bouin's solution for 18-24 hours. The fixed specimens were processed to get 5-7 micrometer thickness paraffin sections. These sections were stained by Haematoxylin and Eosin (H & E), Masson's Trichrome, Periodic Acid-Shiff reagent (PAS) and Alcian Blue, all stain techniques were adapted to that reported by ***Bancroft and Stevens (1990)***. The stained slides were examined and photographed using light research microscope.

To study the histochemistry and ultrastructure of the placenta, 4 apparently healthy pregnant goats were used, they obtained from Mahelt Mossa Station for Reproduction. The goats were slaughtered after 80, 100, 110 and 145 (term) days of gestation. Specimens of placental tissues

including the placentome and intercotyledonary area were taken from the pregnant uteri immediately after slaughter.

Fresh cryostat sections of about 10 micrometers thickness were made and used for demonstration of the activity of the following enzymes :

1. Acid phosphatase (Malatyl, 1971).
2. Alkaline phosphatase (Malatyl, 1971).
3. Succinate dehydrogenase enzyme (Lojda et al., 1979).

For transmission electron microscopy (TEM), small fragments of placentome (0.5-1.0 mm^3) were cut. These tissue fragments were fixed in 2.5% gluteraldehyde buffered with 0.1 M phosphate buffer (pH 7.4) for 12 hours, then washed in several changes of 0.1 M phosphate buffer (pH 7.4) and post fixed in 2% osmium tetraoxide (OsO_4) in phosphate buffer (pH 7.4) for two hours, then dehydrated through ascending grades of ethanol followed by clearing in propylene oxide. The specimens were embedded in an Epon/Araldite mixture in capsules, polymerization was obtained by incubating the capsules in 60 ^oC.

Simithin sections (1 micrometer) were made and stained with toluidin blue for observation and orientation. Ultrathin sections (100 nm) were prepared and routinely stained with uranil acetate and lead citrate. Then examined and EM photographs were taken using Jeol 2003 electron microscope.


RESULTS

RESULTS

According to the developmental changes in the placenta, the specimens were classified into five stages depending on the CVRL (1.5–5 cm, 7–9 cm, 10–13 cm, 16–21 cm and 25–31 cm CVRL).

1. Light Microscope Observation :

1.1. Early stage of placentation and placentome formation (1.5 – 5 cm CVRL) :-

At this stage of development, the definite areas of adhesion between the blastocyst and the uterine epithelium were formed in the pregnant horn. The chorionic epithelium was formed of irregularly stratified layer of cuboidal trophoblasts and darkly stained binucleated giant cells. The trophoblasts has lightly acidophilic, granulated and PAS positive cytoplasm with vesicular, round and centrally located nucleus. The binucleated cells were large in size, round in shape and their cytoplasm was more acidophilic, and contained strongly PAS positive granules, which concentrated in one pole of the cell than the other. These cells appeared in different levels in the trophoblastic epithelium and may be found in the maternal side of placenta (Figs. 1 and 2).

As a result of the contact of chorionic epithelium with the uterine epithelium, two changes in the formation of the placenta were observed; the migration of the binucleated cells from the chorionic

epithelium to the maternal side of the placenta, and the columnar nature of the uterine epithelium was transformed into multinucleated flattened or low cuboidal syncytial plaques (Figs. 1 and 2), which persist until the end of pregnancy.

At 3 cm CVRL, the chorionic epithelium began to proliferate forming small buds of cells (Fig. 3). These buds invade the uterine (caruncular) epithelium, where it form a pit-like invaginations opposite to the chorionic buds (Fig. 4). Latter on (5 cm CVRL), few mesodermal tissue with branches of allantoic vessels invade these chorionic buds forming mesenchymal core, leading to the formation of chorioallantoic villi (Fig. 5). At the same time, the pits were increased in depth forming the maternal crypts.

In the intercotyledonary area, the contact between the chorionic epithelium and the uterine epithelium showed a distinct variation; they were closely contact in some areas, slightly in the others, while at the areas where the uterine glands open, there was no contact between the maternal and the fetal tissues (Fig. 6). At the site of contact, the uterine epithelium was transformed to syncytial plaques either flattened or low cuboidal (Fig. 7) and in some areas complete degeneration of the uterine epithelium was also observed.

1.2. The placenta at 7 cm to 9 cm CVRL :

At this stage of development, the primary formed villi increased in size and became branched forming secondary villi which increased in

length and extent deeply in the maternal tissue and rebranched to form small tertiary ones (Fig. 8).

The lining epithelium of the primary villi (stem of the villi) formed of more than one layer of trophoblasts and giant cells. While that of the secondary and tertiary villi formed of only one layer of these cells, as the trophoblasts predominant with few giant cells in between (Figs. 9, 10).

The connective tissue core in the villi was formed of cellular and fibrous elements. The fibrous element was formed of fine collagen fibers which gave slightly positive reaction with PAS and strong positive reaction with alcian blue. The cellular element was composed of fibroblasts and undifferentiated mesenchymal cells with different shapes and sizes. The fibroblasts have elongated, vesicular nuclei, with lightly acidophilic cytoplasm, which gave slightly positive reaction with PAS. These cells have many elongated cytoplasmic processes (Figs. 9, 11).

The maternal crypts lined by one layer of irregular cells with indistinct cell membrane. They have round, darkly basophilic nuclei and moderate PAS positive cytoplasm. The connective tissue septa formed mainly of PAS positive collagen fibers with few fibroblasts and polyhedral cells (Fig. 12).

In the intercotyledonary area, the chorionic epithelium was formed of irregularly stratified epithelium of low columnar and few binucleated giant cells in between (Fig. 13). On the other hand, the uterine epithelium was regenerated and became tall columnar cells.

1.3. The placenta at 10 – 13 cm CVRL :

At this stage of development, the placenta became large in size with more branched villi than the previous stages. While the lining epithelium of the different villous branches was still unchanged. It was more than one layer in the primary villi and only one layer in the secondary and tertiary ones (Fig. 14).

The collagen fibers in the connective tissue core increased in amount and became more PAS positive than the previous stages. The cells became to aggregate near the trophoblastic basement membrane (Figs. 15, 16). Wide blood spaces appeared between the connective tissue core and the villous epithelium. They were lined by on layer of endothelial cells and contain blood elements (Fig. 17).

1.4. The placenta at 16–21 cm CVRL :

At this stage of the placental development, the placentome reach its maximal size. The villi increased in length and extent to the end of the placentome (Fig. 18). The lining epithelium of the primary villi was similar to the previous stages of development. At the tip of the villi, the giant cells increased in number. These giant cells either mononucleated or binucleated and contain PAS positive granules (Fig. 19). Some binucleated giant cells showed different degrees of degenerative changes in both cytoplasm and nucleus (Fig. 20). Cytoplasmic degranulation and vacuolation and subsequently decrease in the cytoplasmic PAS positive

reaction were the common results of degenerative changes in the cytoplasm, besides, degeneration by nuclear pyknosis was encountered.

At this stage, the maternal septa reduced in size and showed moderately PAS positive reaction (Fig. 21). The cryptal epithelium symcytium have comparatively large, spherical and hyperchromatic nuclei, and rested on a PAS positive basement membrane (Fig. 22). The maternal septa composed mainly of collagen fibers and fibroblasts (Fig. 23).

1.5. The placenta at 25-31 cm CVRL (full term) :

At this more advanced stage, the fetal villi and corresponding maternal crypts did not increase in length, but divided repeatedly thus increasing the area of apposed surface epithelia mediating physiological exchange between mother and fetus. The number of the binucleated cells decreased in number and some of them showed signs of degeneration. The blood spaces near the basement membrane of the fetal trophoblasts increased in size and number. The fetal and maternal tissue appeared to be more contact than the previous stages. The syncytium cover the maternal crypts became more flattened than the previous stages.

1.6. Morphological structure of ripe placenta :

The does (she-goat) placenta appeared to be formed of two distinct components; the placentomes and intercotyledonary areas. The placentome consisted of a tuft of fetal villi (cotyledons) intimately enmeshed with maternal crypts (caruncles) (Fig. 24).

Grossly, the placentomes were arranged in four rows, two dorsally and two ventrally. The average number of the placentome in the pregnant uterus was 126 placentomes. The ripe placentome was button in shape with central concavity, in which the cotyledons were fastened in the formed concavity. They were either rounded or somewhat elongated and became more flattened and long as gestation advanced (Fig. 25).

The cotyledon consisted of numerous, nearly slender and richly vascular villi. These villi were wide more branched near the surface and narrow less branched in the depth of the placentome (Fig. 26). Each villous was composed of a vascular mesenchymal core covered by a layer of two cell types rested on a distinct PAS positive basement membrane (Fig. 27). The first cell type was cuboidal or polyhedral in shape. Their cytoplasm was light acidophilic. Their nuclei were relatively large, regular spherical in shape, central in position and stained light basophilic with coarse chromatine. The second cell type was comparatively few in number and scattered in between the first type. They were nearly round in outline or might show variable degrees of irregularity. Their cytoplasm was highly acidophilic and granular. The cytoplasmic granules were fine and gave positive reaction with PAS. They may be mononucleated, binucleated or even trinucleated. The nuclei were large regular round, central or eccentric in position and stained light basophilic with coarse chromatin.

The mesenchymal tissue, that filled the villous core was of embryonic connective tissue contained wide blood vessels (Fig. 28).

The maternal crypts were lined by a layer of flattened or low cuboidal cells with indistinct cell boundary, their cytoplasm was highly acidophilic and showed PAS positive reaction. Their nuclei were round, oval or even elongated according to the shape of the cell (Fig. 29).

The maternal septa were composed of fibrous and cellular elements. The fibrous element was formed mainly of collagen fibers which gave a strong positive reaction with PAS. The predominant cell types were of fibroblasts, undifferentiated mesenchymal cells and lymphocytes (Fig. 30).

In the intercotyledonary area, the chorionic epithelium was simply apposed to the endometrium; it was formed of a single layer of high cuboidal trophoblasts with few binucleated cells scattered in between. The number of the binucleated cells was less than that found in the epithelium of the villi. The uterine epithelium in this region was composed of a layer of ciliated columnar cells (Fig. 31).

2. Fine structure of the cells of the placentome :

2.1. Trophoblast :

The cell membrane of the trophoblast had many irregular invaginations especially at the cell side adjacent to the syncytium (Fig. 32). Membrane-bound vesicles were observed near these invaginations. The cytoplasm contained a moderate number of mitochondria, they were rounded in shape with parallel cristae (Fig. 33). The Golgi apparatus was inconspicuous. Rough endoplasmic reticulum in form of wide cisternae, and polysomes in form of rosettes were scattered throughout the cytoplasm. Their nuclei were relatively large, rounded and have one or two nucleoli and clumped chromatin (Fig. 34).

2.2. Binucleated giant cells :

The binucleated cells were considerably larger than the cuboidal trophoblasts (Fig. 35). They were polyhedral in shape. They have two unequal size nuclei, which occupy most of the cytoplasm. Like cuboidal trophoblast, the cytoplasm of the binucleated cells contain numerous dilated cisternae of granular endoplasmic reticulum, mitochondria and a variable number of free ribosomes in the ground cytoplasm. Electron dense, small exocytotic vesicles were seen near the binucleated giant cells.

2.3. Syncytial layer :

The surface of the syncytial layer was provided with irregular microvilli which fitted into the corresponding invaginations of the plasma

membrane of the trophoblastic cells (Figs. 33, 36). The nuclei were electron dense, elongated or slightly irregular in shape and contain inconspicuous elctron dense marginal chromatin. The cytoplasm was more electron dense than the trophoblastic cells. It contained numerous free ribosomes, round or elongated mitochondria and membrane bound, light vesicles (Fig. 37).

2.4. The maternal septal tissue :

It was formed of fibroblasts-like cells and collagenic fibers. The fibroblasts were of two types. The first have high electron dense cytoplasm, while the second type have light cytoplasm (Fig. 38). Between the fibroblasts there were relatively large fat globules.

3. Enzyme activity :

3.1. Acid phosphatase :

Strong acid phosphatase activity was observed in the binucleated cells, in the chorionic villi and the maternal septa. While a moderate activity was seen in the cryptal syncytium and weak to moderate activity in the trophoblast cells of the chorionic epithelium. The acid phosphatase activity was weak to negative in the other tissue components (Fig. 39).

3.2. Alkaline phosphatase :

Intense granular alkaline phosphatase activity was found in the perinuclear areas of the trophoblast cells. Negative or very weak activity was observed in the binucleated giant cells and in the mesenchymal core and its mesenchymal cells (Fig. 40). The cryptal cells showed moderate to strong alkaline phosphatase activity, while the septae were moderately positive. High alkaline phosphatase activity was found in the wall of the blood vessels of the maternal septae (Fig. 41).

3.3. Succinate dehydrogenase :

Intense granular succinate dehydrogenase activity was observed in the cryptal cells and periphery in the binucleated giant cells. Moderate activity was observed in the trophoblast cells. The maternal septae were weakly positive while the mesenchymal core and mesenchymal cells of the chorionic villi showed no activity (Fig. 42).

Table (1) : Carbohydrate distribution in the placenta of the goat as demonstrated by periodic schiff's reagent and alcian blue stain.

Stain	Fetal side			Maternal side	
	Chorionic ep.		Mesenchymal core	Cryptal ep.	Maternal septa
	Trophoblast	Binucleated cells			
1) Periodic acid Schiff reagent (PAS)	-/+	+++	+	++/+++	+++
2) Alcian blue	+	-/+	+++	-/+	+

- = negative
+ = weak activity
++ = moderate activity
+++ = strong activity

Table (2) : Histoenzymological observation in the placenta of the goat

Enzyme	Fetal side			Maternal side	
	Chorionic ep.		Mesenchymal core	Cryptal ep.	Maternal septa
	Trophoblast	Binucleated cells			
1) Acid Phosphatase	+/++	+++	-/+ Mesenchymal cell	++/+++	+++
2) Alkaline phosphatase	+/++	-/+	+	++/+++	++/+++ wall of bl.v.
3) Succinate dehydrogenase	++	+++	-	++	+

\- = negative
\+ = weak activity
++ = moderate activity
+++ = strong activity

Plate (1) A histogram showing PAS and Alcian blue distribution in the placenta of goat.

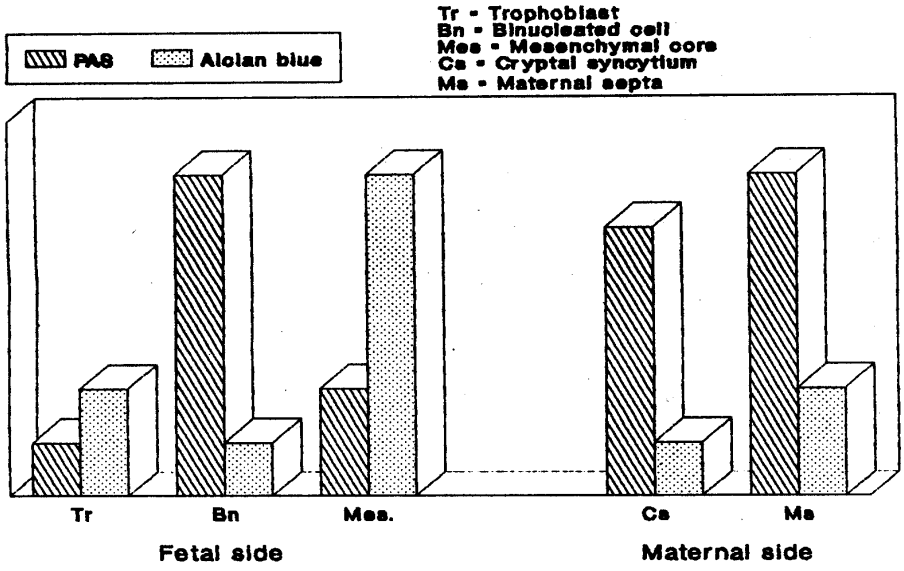


Plate (2) A histogram showing some enzyme activity in the placenta of goat

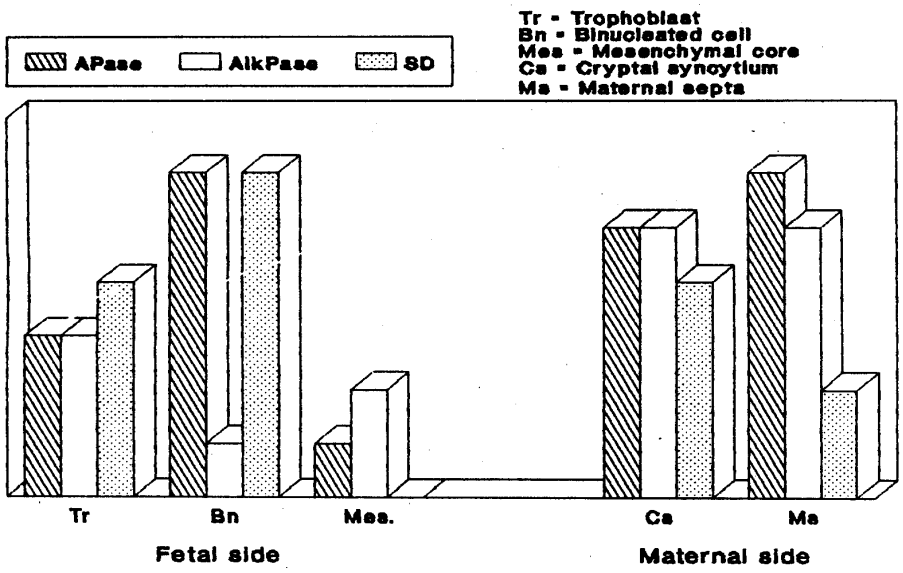


Fig. (1) Placenta of the goat at (1.5 CVRL) showing :

A : Allantoic blood vessels.

T : Trophoectodermal layer.

Bn : Binucleated cells (PAS +ve).

Tr : Trophoblastic cuboidal cells.

Su : Subepithelial connective tissue.

(PAS technique, X 40).

Fig. (2) Placenta of the goat at (1.5 CVRL) showing :

T : Trophoectodermal layer.

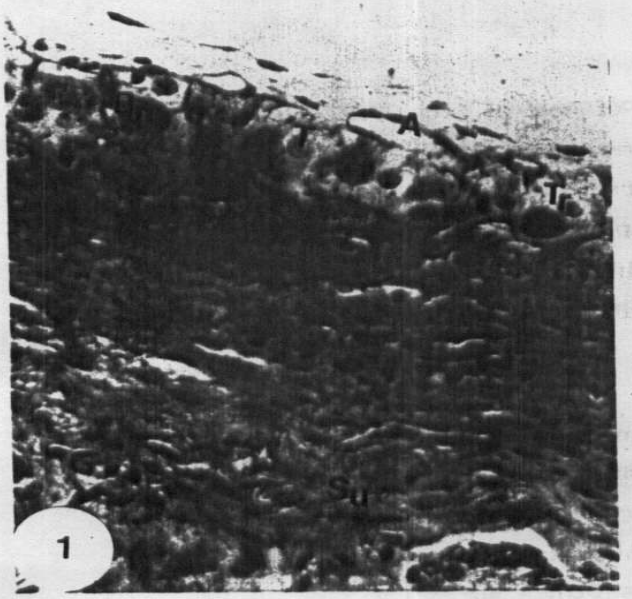
Bn : Binucleated cell.

Ut : Transformed uterine epithelium.

Mb : Migrating binucleated cells.

Su : Subepithelial connective tissue.

(H & E Stain, X 40).


A
Tr
Su
1

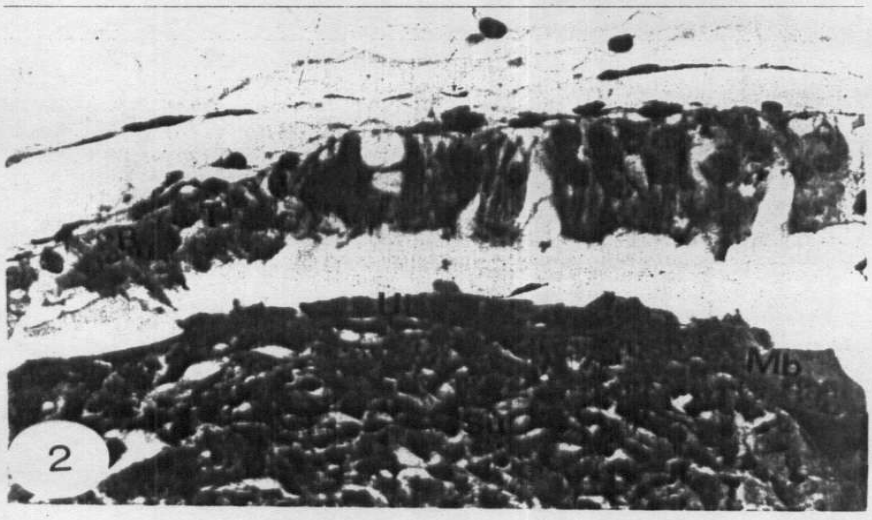

Mb
2

Fig. (3) Placenta of goat at 3 cm CVRL showing :

T : Trophoectoderm layer.

Mc : Maternal crypt.

Su : Subepithelial connective tissue.

Ug : Uterine gland.

(H & E Stain, Obj. X 10)

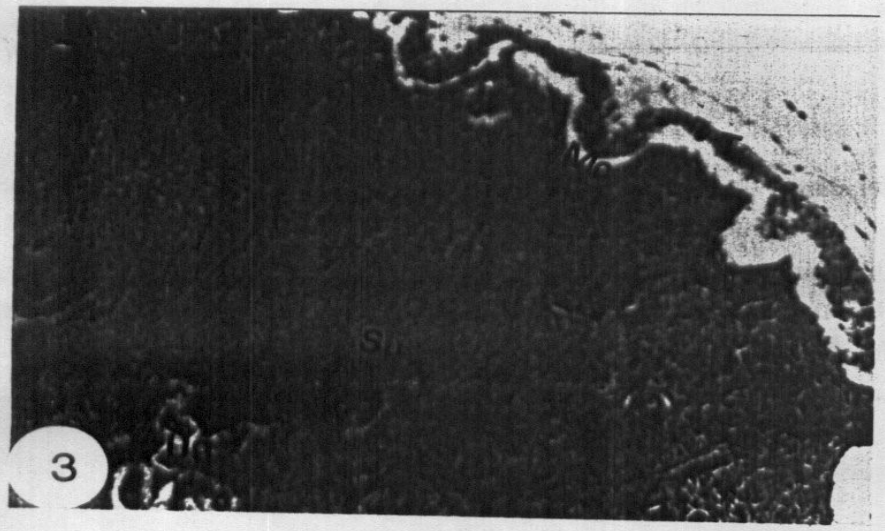
Fig. (4) High magnification of Fig. (3) showing :

Bn : Binucleated cells in chorionic bud.

Mg : Lining of maternal crypts.

Su : Subepithelial connective tissue.

(H & E Stain, Obj. X 40)



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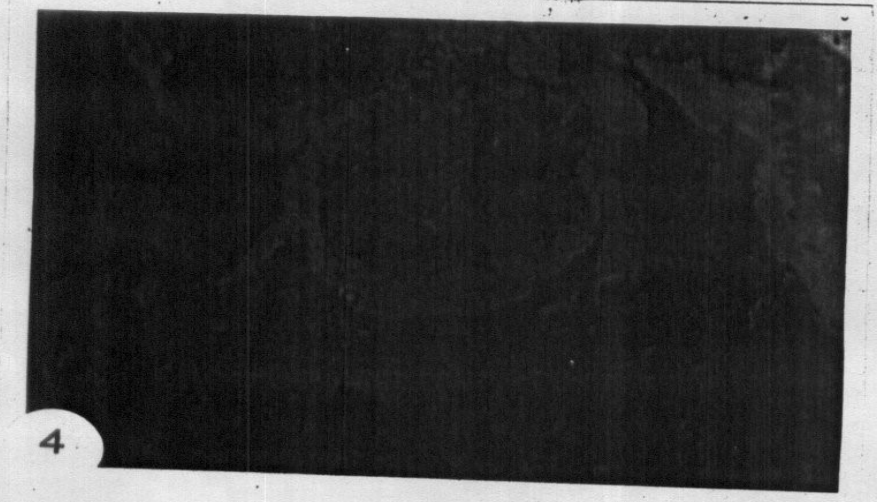


4

Fig. (5) Placenta of goat at 5 cm CVRL showing :

V : Chorionic villi.

Bn : Binucleated cells.

Db : Degenerated binucleated cell.

Ms : Maternal septa.

(H & E Stain, Obj., X 40).

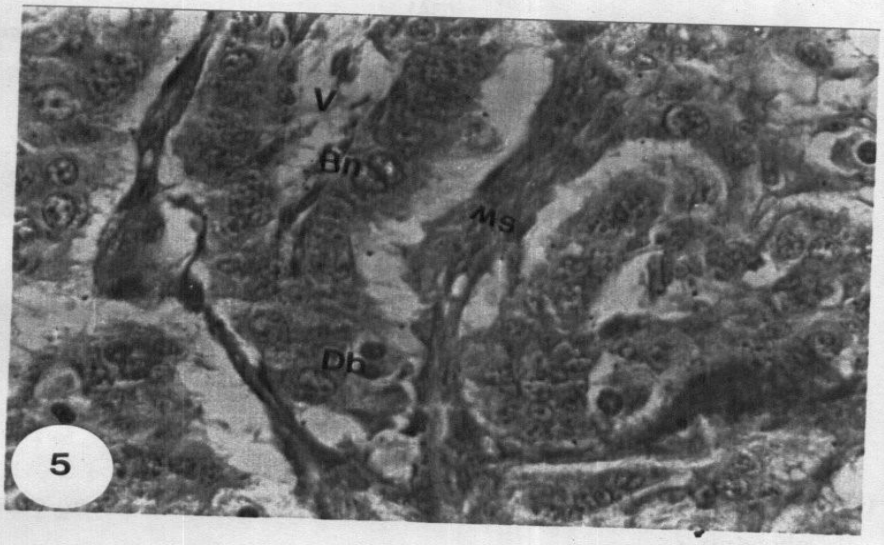

V
Bn
Ms
Db
5

Fig. (6) Intercotyledonary area of placenta of goat at 3 cm CVRL showing :

U_g: Uterine gland.

T : Trophoectoderm layer.

Su : Subepithelial connective tissue.

(H & E Stain, Obj., X 10).

Fig. (7) Intercotyledonary area of placenta of goat at 3 cm CVRL showing :

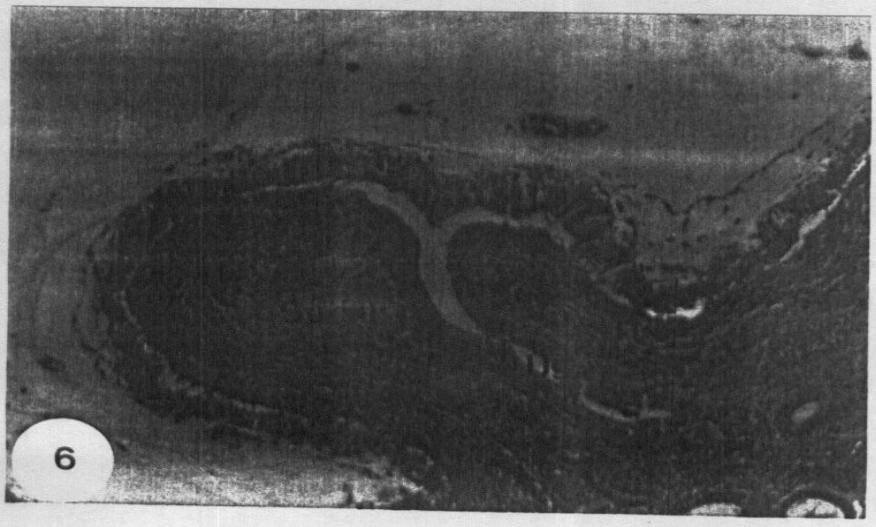
T : Trophoectodermal layer.

U_g: Uterine gland.

Du : Degenerated uterine epithelia.

M : Myometrium.

(H & E Stain, Obj., X 10)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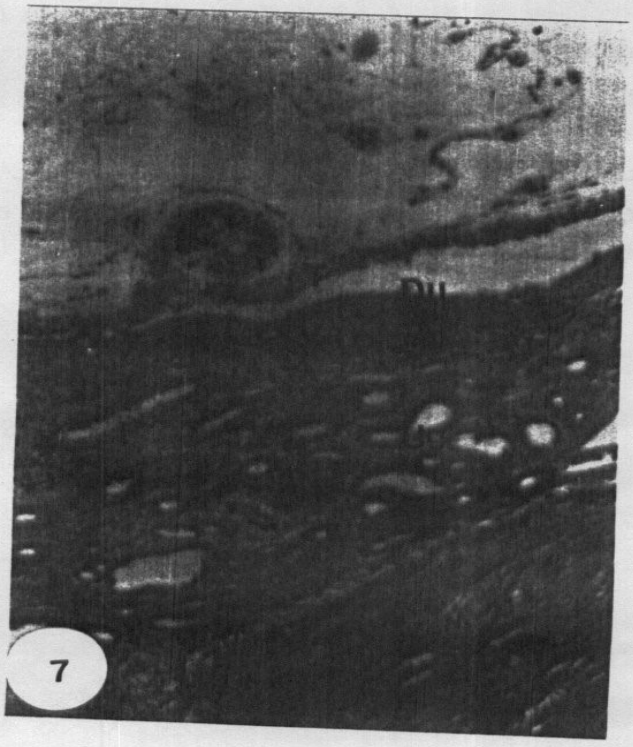

7

Fig. (8) Placentome of goat placenta at 7 cm CVRL showing :

A : Allantoic blood vessel.

Vp : Primary villous.

Vs : Secondary villous.

Vr : Tertiary villous.

Ms : Maternal septa.

(H & E Stain, Obj. X 4)

Fig. (9) The chorionic villous of goat placenta at 7 cm CVRL showing :

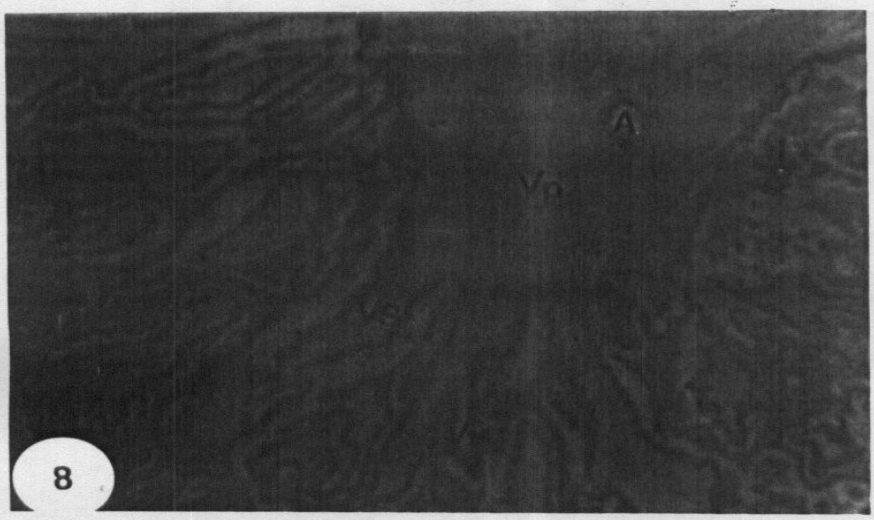
Mc : Mesenchymal connective tissue core.

F : Fibroblasts

Bn : Binucleated cell.

Ct : Cuboidal trophoblasts.

(H & E Stain, Obj., X 40)


A
Vp
8

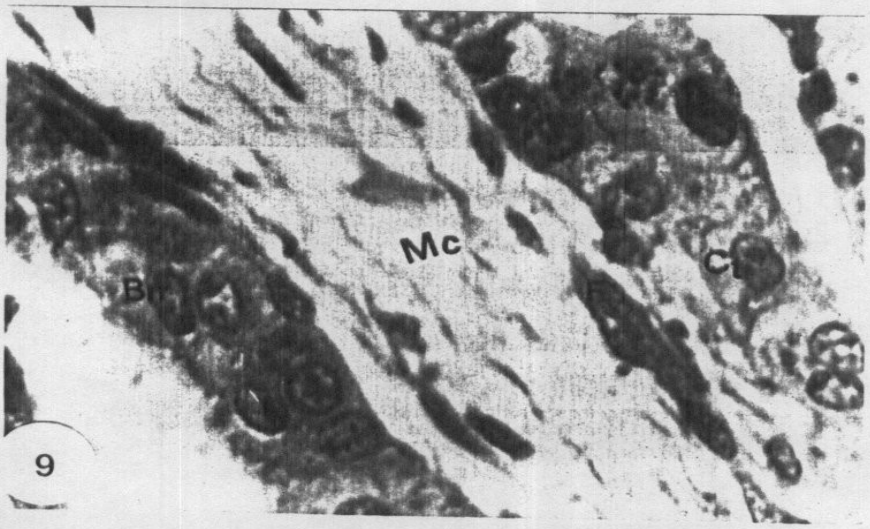

Mc
9

Fig. (10) Placenta of goat at 7 cm CVRL showing the lining epithelium of the chorionic villus.

Tg : Trinucleated giant cell (PAS + ve).

Gt : Cuboidal trophoblast.

Bm : Basement membrane.

Mc : Mesenchymal core.

(PAS technique, Oil immersion)

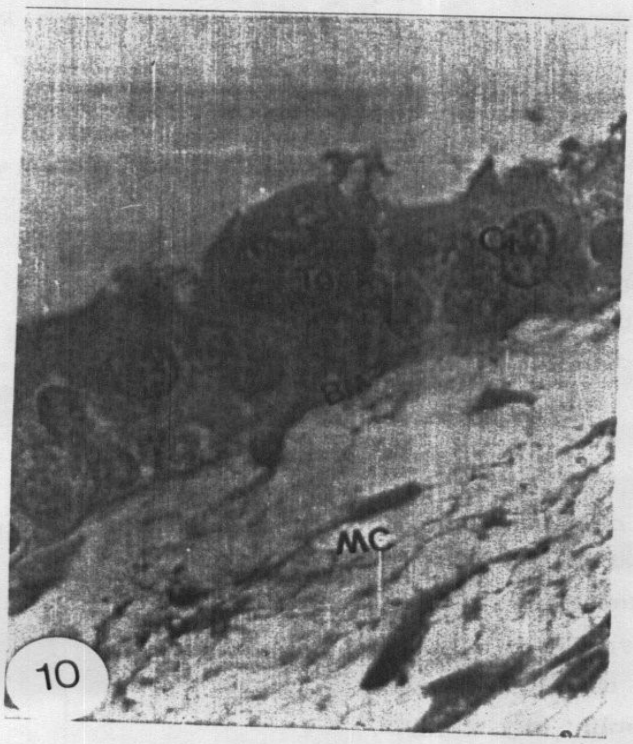

MC
10

Fig. (11 a) Connective tissue core of a villous of goat placenta at 7 cm CVRL showing :

A : Allantoic blood vessel.

E. Endothelium.

F : Fibroblast.

T. trophoectoderm.

(H & E Stain, Obj., X 40)

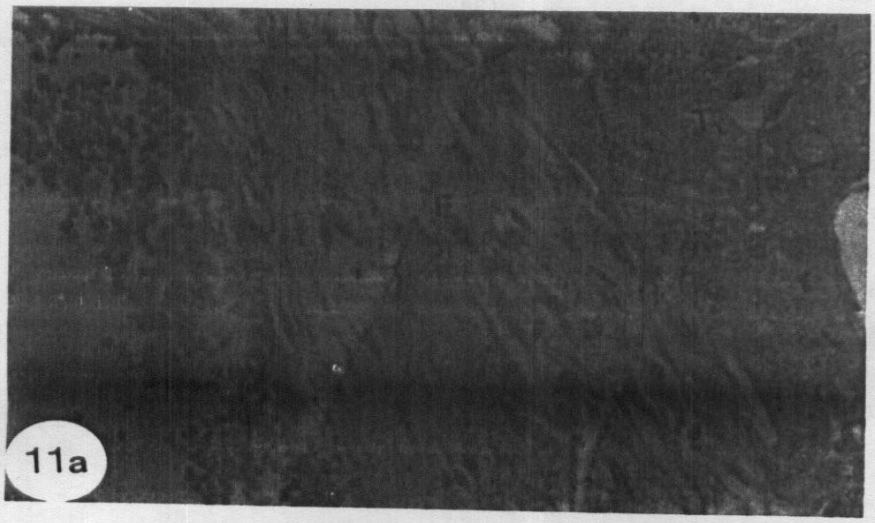
Fig. (11 b) Placenta of goat at 8 cm CVRL showing the alcianophilic mesenchymal core.

T : Trophoectoderm.

Mc : Mesenchymal core (Alcian blue +ve)

Ms : Maternal septa.

(Alcian blue stain, Obj. X 10)



11a

11b

Fig. (12) Placenta of goat at 9 cm CVRL showing :

Cs : Cryptal syncytium.

Bn1 : Binucleated cell type 1.

Bn2 : Binucleated cell type 2.

Ms : Maternal septa.

T : Trophoectodermal layer.

(PAS technique, Obj. X 40).

Fig. (13) Intercotyledonary area of the goat placenta at 7 cm CVRL showing :

T : Trophoectodermal layer.

Ue: Uterine epithelium

Su : Subepithelial layer.

M : Myometrium.

(PAS technique, Obj. X 40).


Br
T
T
Me
12

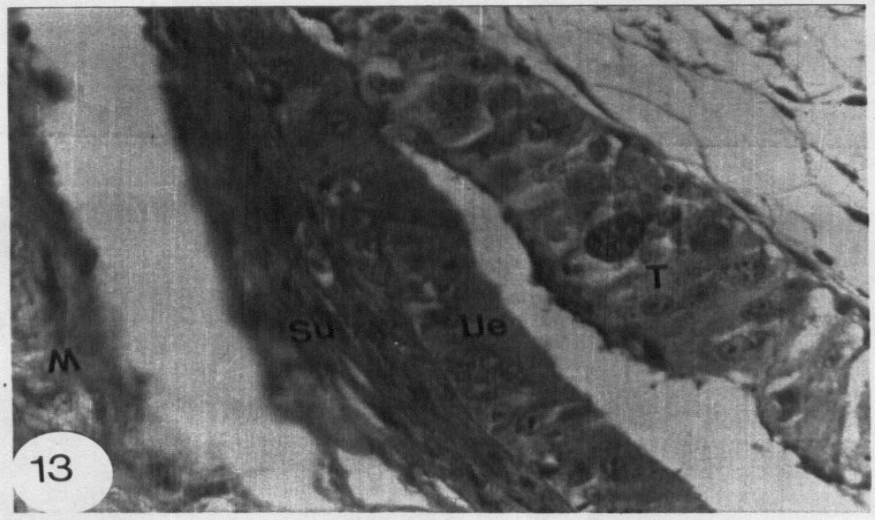

T
Su
Ue
M
13

Fig. (14) Placenta of goat at 10 cm CVRL showing :

Vp : Primary villous.

Vs : Secondary villous.

Ms : Maternal septa.

(H & E Stain, Obj. X 10)

Fig. (15) Mesenchymal connective tissue core of a villous of goat placenta at 11 cm CVRL showing :

T : Trophoectoderm.

F : Fibroblast.

Bm : Basement membrane.

(H & E Stain, Oil immersion).


Vp
Vs
14

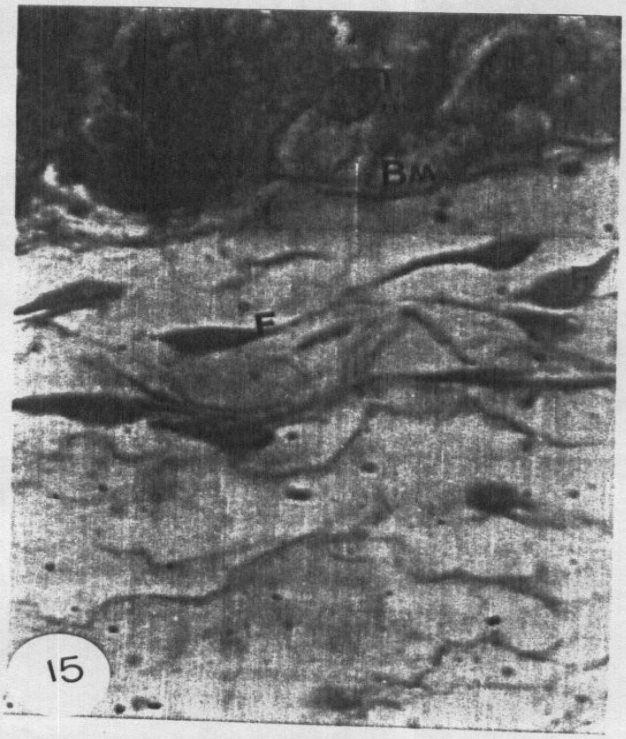

BM
F
15

Fig. (16) : Mesenchymal connective tissue core of a villous of goat placenta at 11 cm CVRL showing :

T : Trophoectoderm

F : Fibroblast.

Bn : Binucleated cell in the mesenchymal connective tissue.

(H & E Stain, Oil immersion)

Fig. (17) Placenta of goat at 13 cm CVRL showing :

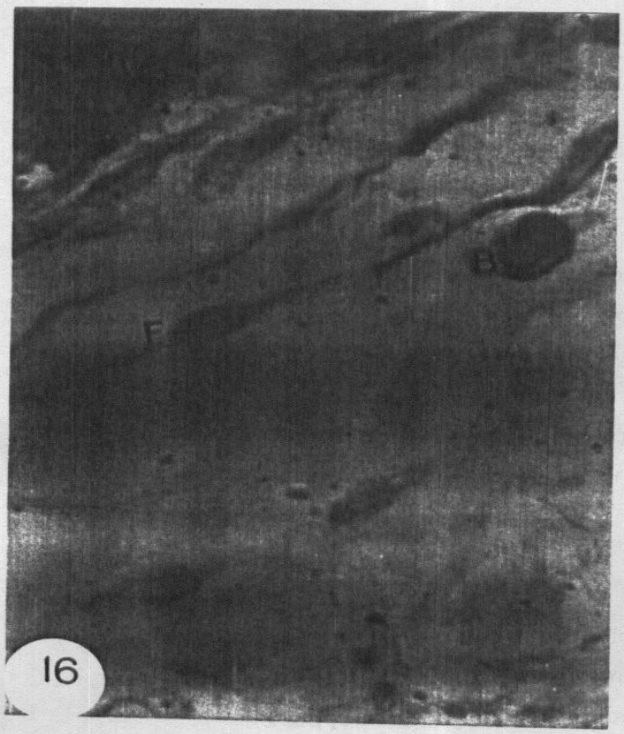
A : Allantoic blood vessels under the basement membrane.

T : Trophoectodermal layer.

E : Endothelium.

Ms : Maternal septa.

(H & E STAIN; Obj. X 40).



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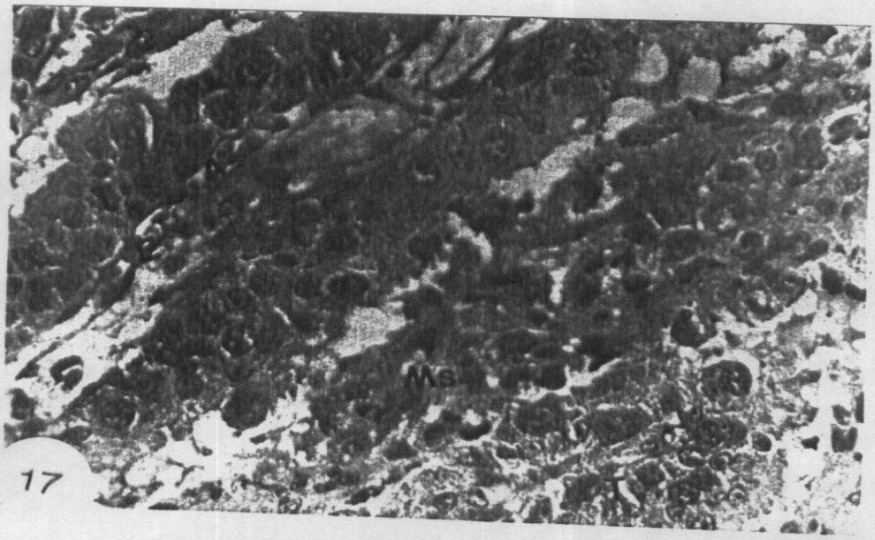


17

Fig. (18) Placenta of goat at 16 cm CVRL showing :

Cv : Chorionic villi.

T : Trophoectoderm.

Cs : Cryptal syncytium.

Ms : Maternal septa.

Su : Subepithelial connective tissue layer.

(PAS technique, Obj. X 40).

Fig. (19) Placenta of goat at 16 cm CVRL showing :

Bn : Binucleated giant cell (PAS +ve)

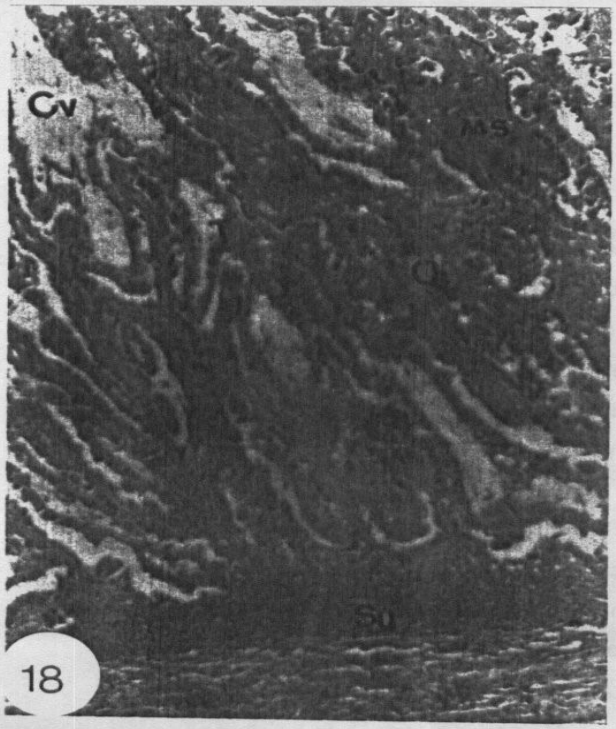
Mn : Mononucleated giant cell (PAS +ve)

Tn : Trinucleated giant cell (type 2)

Cs : Cryptal syncytium.

Ms : Maternal septa.

(PAS technique, Oil immersion)



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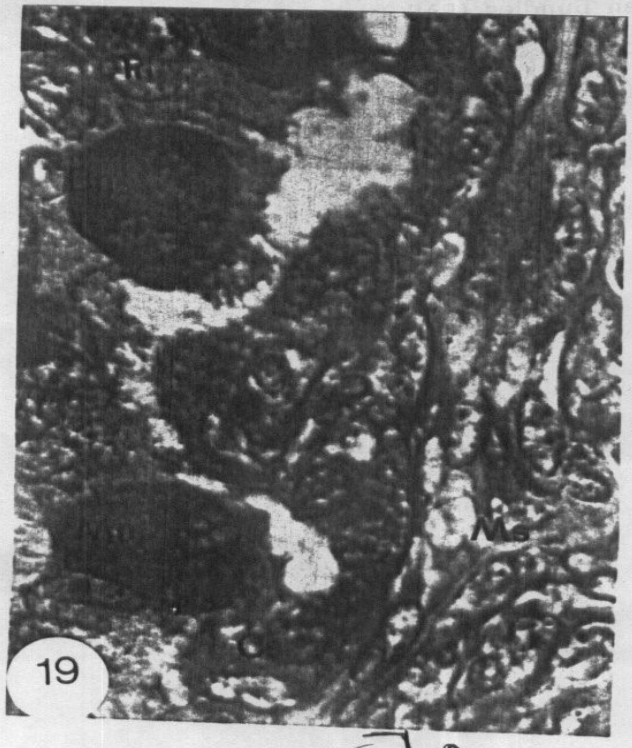


19

Fig. (20) Maternal septa of goat placenta at 16 cm CVRL showing :

F : Fibroblasts

Co : Collagen bundles (PAS +ve).

Dgnd : Dgenerated binucleated cell.

Tr : Trophoblasts.

(PAS technique, Oil immersion).

Fig. (21) Placenta of goat at 20 cm CVRL showing the Maternal septa in btwen the trophoctodermal layrs.

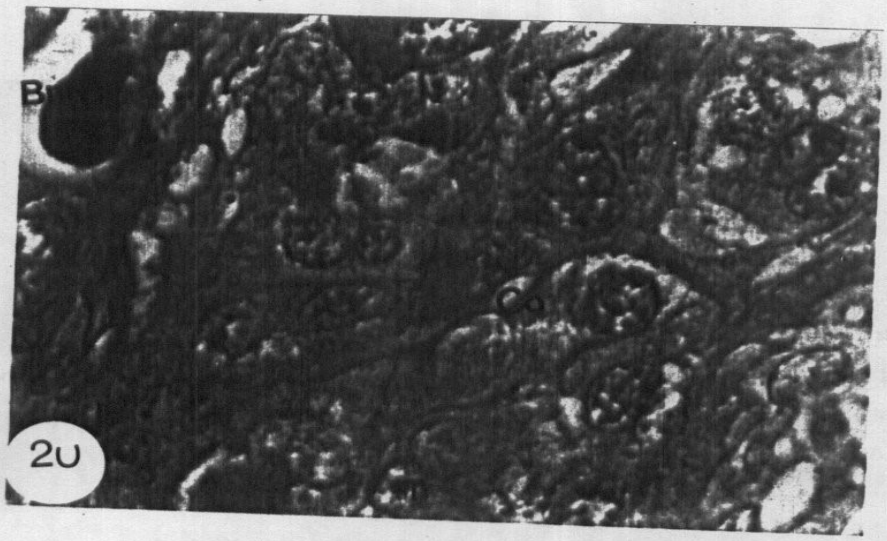
T : Trophoectoderm

Cs : Cryptal syncytium.

Co : Collagen bundles (PAS +ve)

Mc : Mesenchymal connective tissue core.

(PAS tchnique, Obj. X 40).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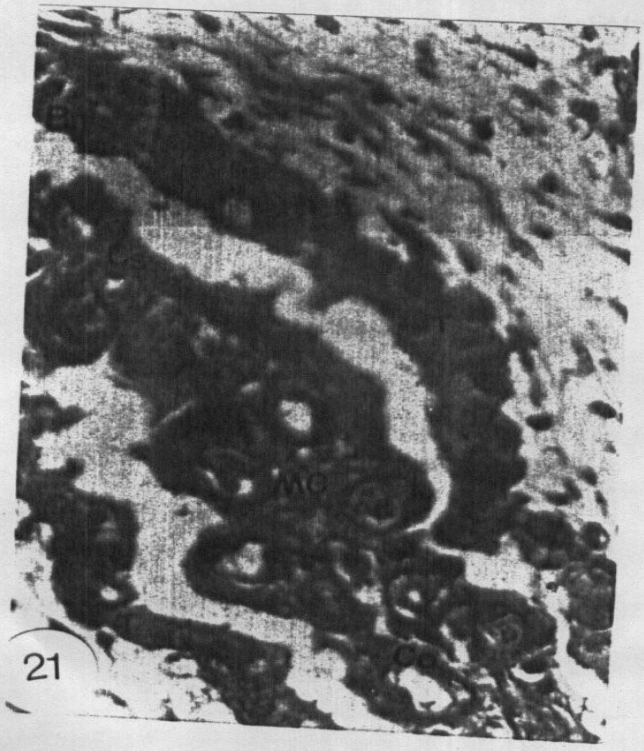

21

Fig. (22) Maternal septa of goat placenta at 21 cm CVRL, lined by cryptal syncytium in between the trophoectodermal layers.

Tr : Trophoblasts.

Bn : Binucleated cells (PAS +ve)

Cs : Cryptal syncytium.

Ms : Maternal septa.

(PAS technique, Oil immersion).

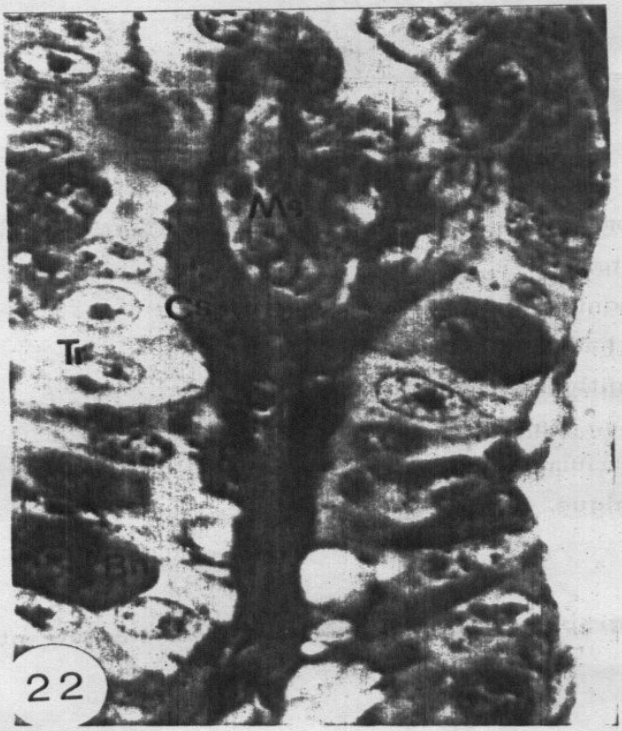
Fig. (23) Goat placenta at 21 cm CVRL showing :

Co : Collagen bundles.

T : Trophoectoderm.

Mc : Mesenchymal connective tissue core.

(Masson's Trichrom stain, Obj. X 40)



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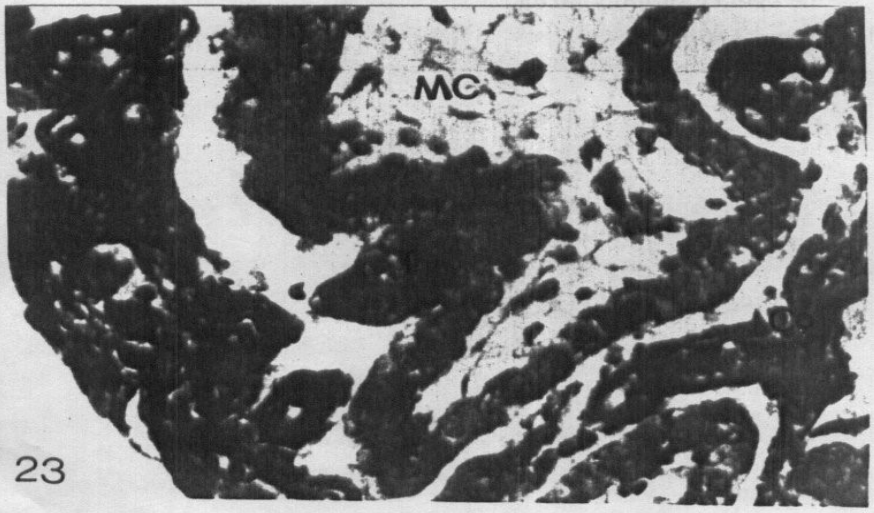


23

Fig. (24) Placentome of the goat placenta at 7 cm CVRL showing :

P : Placentome

Cv : Chorionic villi.

Ms : Maternal septa.

Su : Subepithelial connective tissue.

Ug : Uterine gland.

M : Myometrium.

(PAS technique, Obj. X 4).

Fig. (25) A photograph of the placentome of goat placenta at 10 cm CVRL.

N : Neck

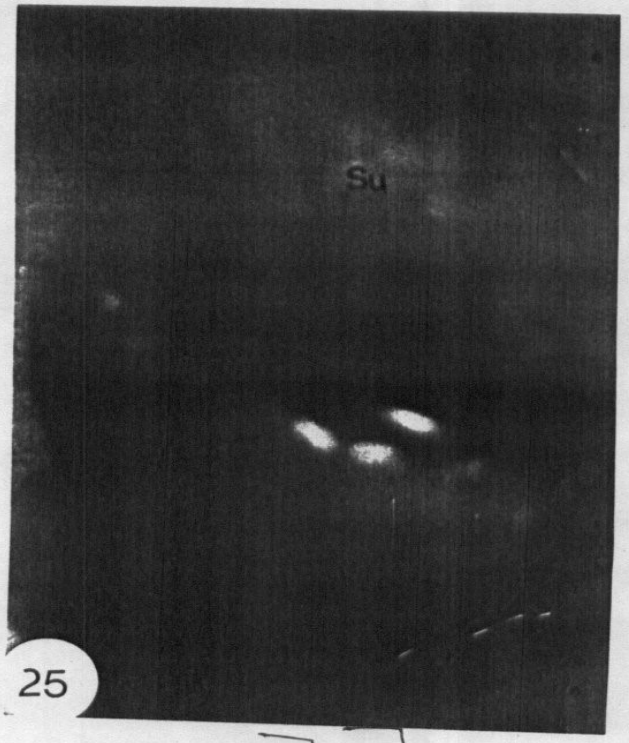
Su : Surface

Sc : Concavity (depression)

I : Intercotyledonary area

M : Myometrium

24



25

Fig. (26) Placenta of goat at 18 cm CVRL showing :

Vp : Primary chorionic villi.

Vs : Secondary chorionic villi.

Ms : Maternal septa.

Su : Subepithelial connective tissue.

(PAS technique, Obj. X 4)

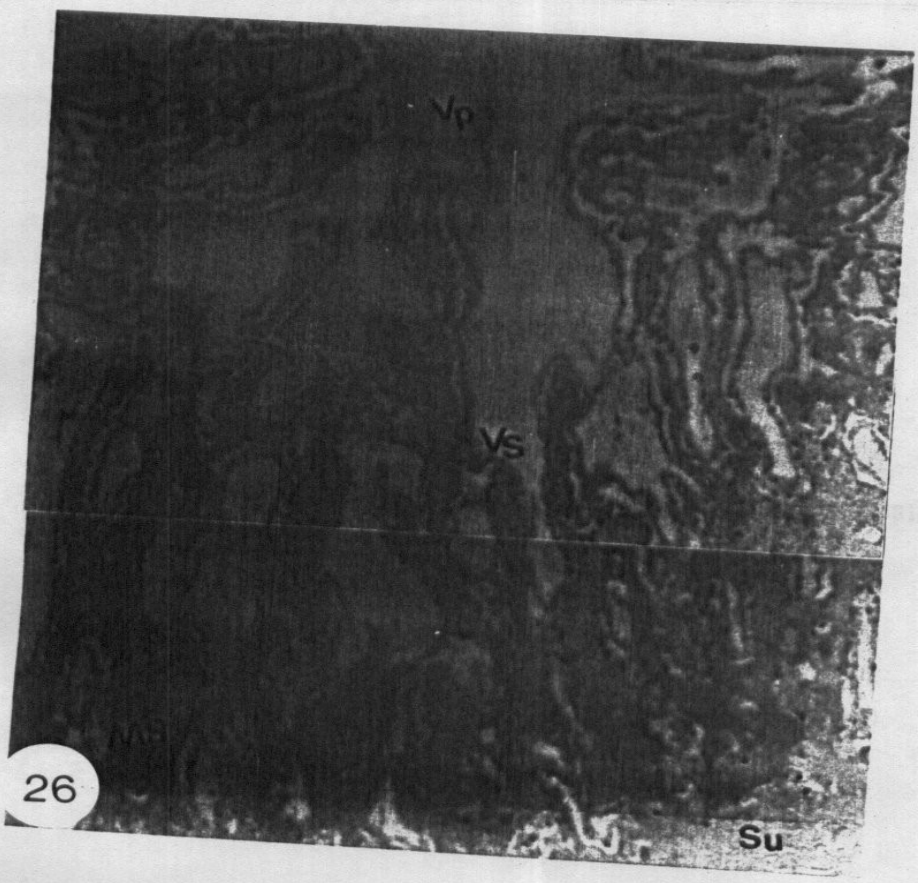

Vp
Vs
26
Su

Fig. (27) Chorionic villi of ripe placenta of goat showing :

Mc : Mesenchymal connective tissue core.

Bn1 : Binucleated giant cell (type 1)

Bn2 : Binucleated giant cell (type 2)

Cs : Cryptal syncytium.

Ms : Maternal septa.

(PAS technique, Obj. X 40).

Fig. (28) Mesenchymal core of a villous of goat placenta at 16 cm CVRL.

F : Fibroblast.

A : Blood vessel (allantoic)

T : Trophoectoderm.

(H & E Stain, Obj. X 40)

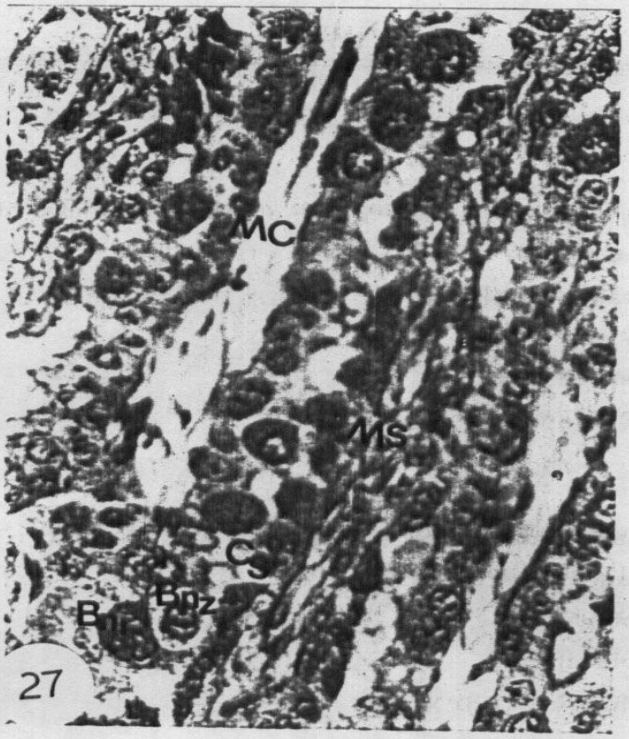


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Fig. (29) Cross section of placentome of goat placenta at 21 cm CVRL showing :

Cy: Cryptal syncytium.

T : trophoectoderm.

Ms : Maternal septa.

(PAS technique, Obj. X 10)

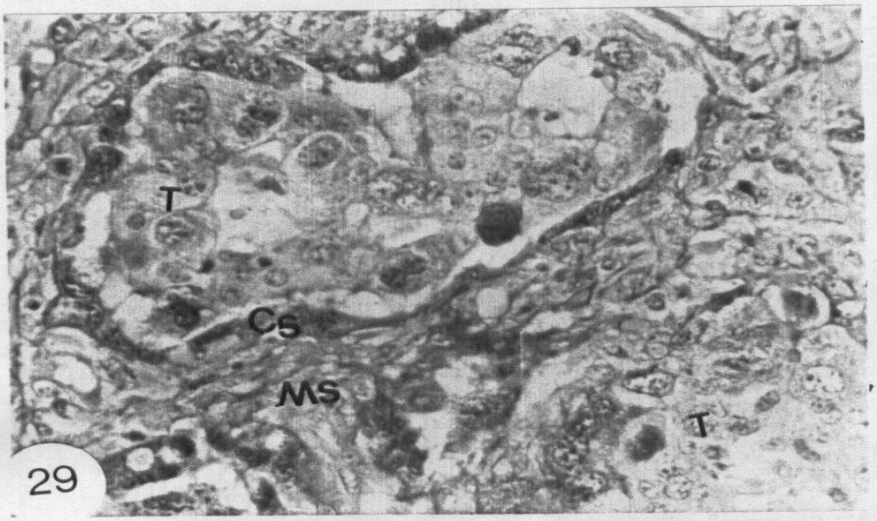
Fig. (30) Maternal septa of ripe placenta of goat showing :

Co : Collagen fibers (PAS +ve)

L : Lymphocyte.

F : Fibroblasts.

(PAS technique, Obj. X 40)



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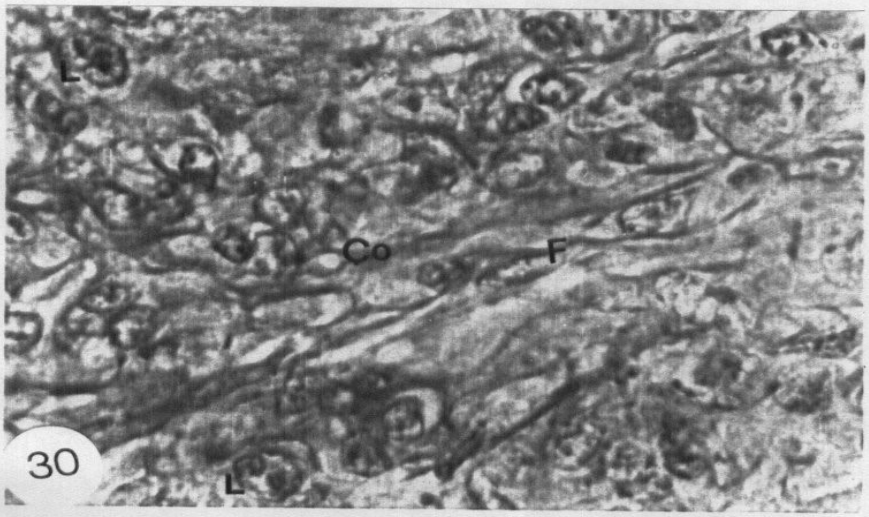

30

VC

Fig. (31) Intercotyledonary area of the goat placenta at 21 cm CVRL showing :

T : Trophoectodermal layer.

Ue : Uterine epithelium.

Su : Subepithelial connective tissue

Mb : Maternal blood vessel.

(PAS technique, Obj. X 40).

Fig. (32) Electron micrograph of goat placenta at late pregnancy showing the microvillus interdigitation (Mv) between the cryptal syncytium and trophoblast (X 10.000).

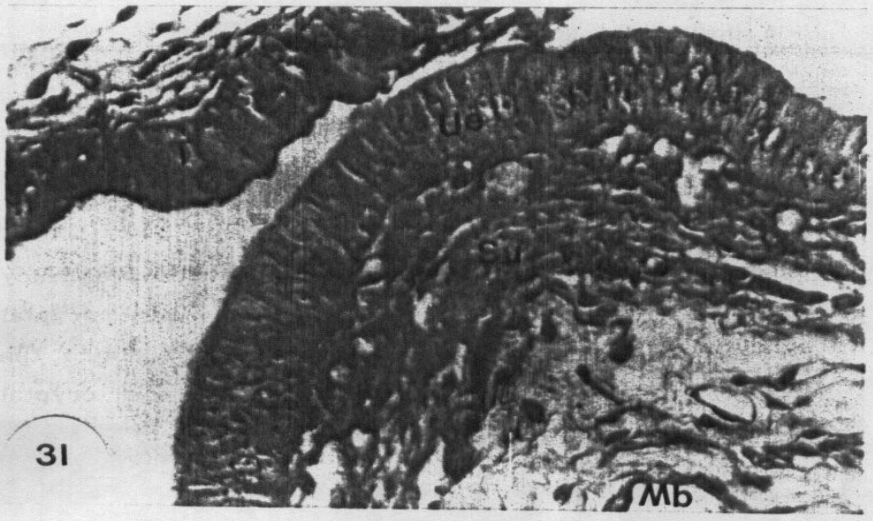

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Fig. (33) Electron micrograph of goat placenta at late pregnancy showing, Trophoblast (T_T) interdigitated with cryptal syncytial layer (Cs) through microvilli (Mv). Notice the nucleus of the trophoblast (Nt) and of the cryptal syncytium (Nc) (X 13.000).

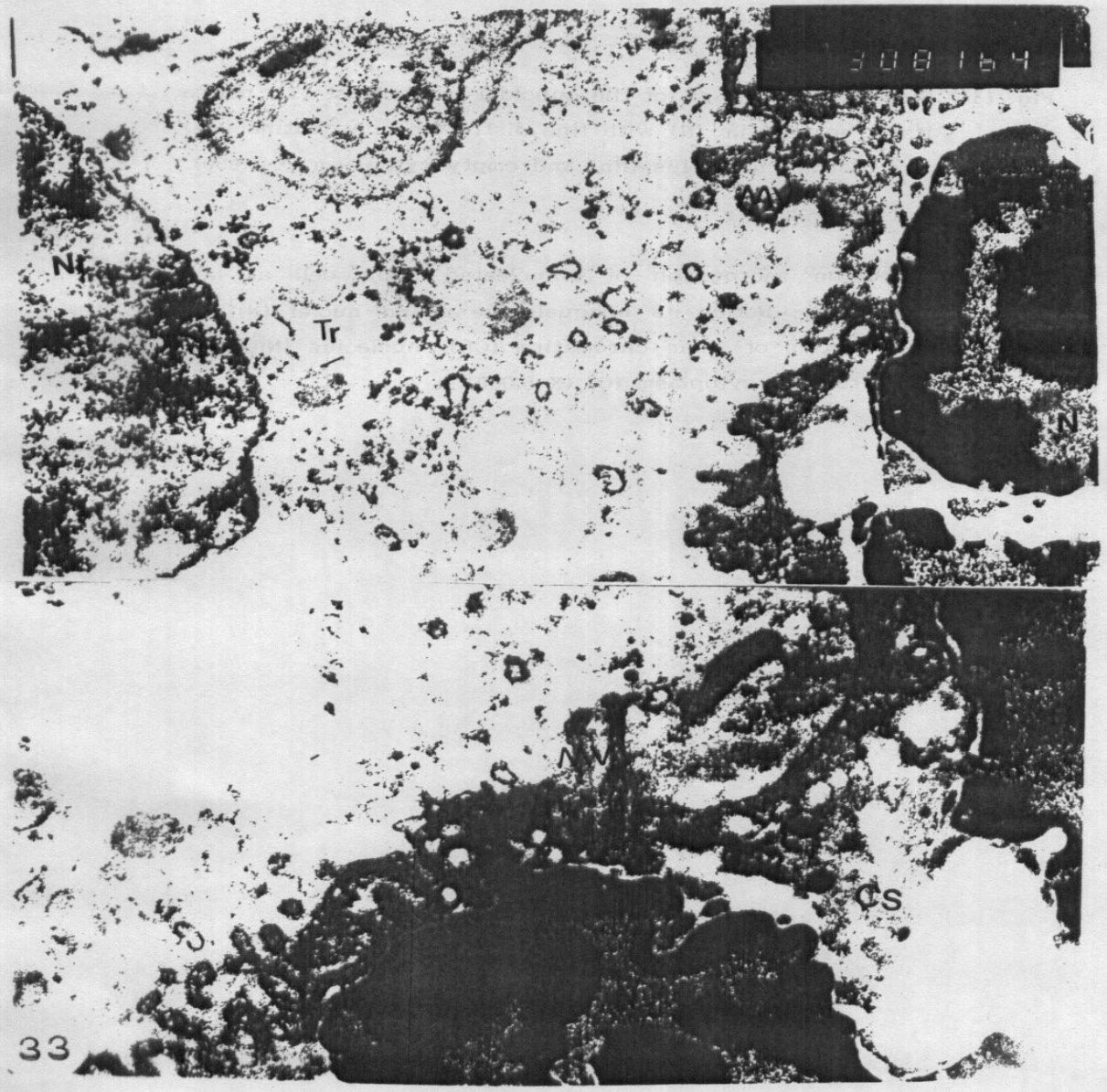
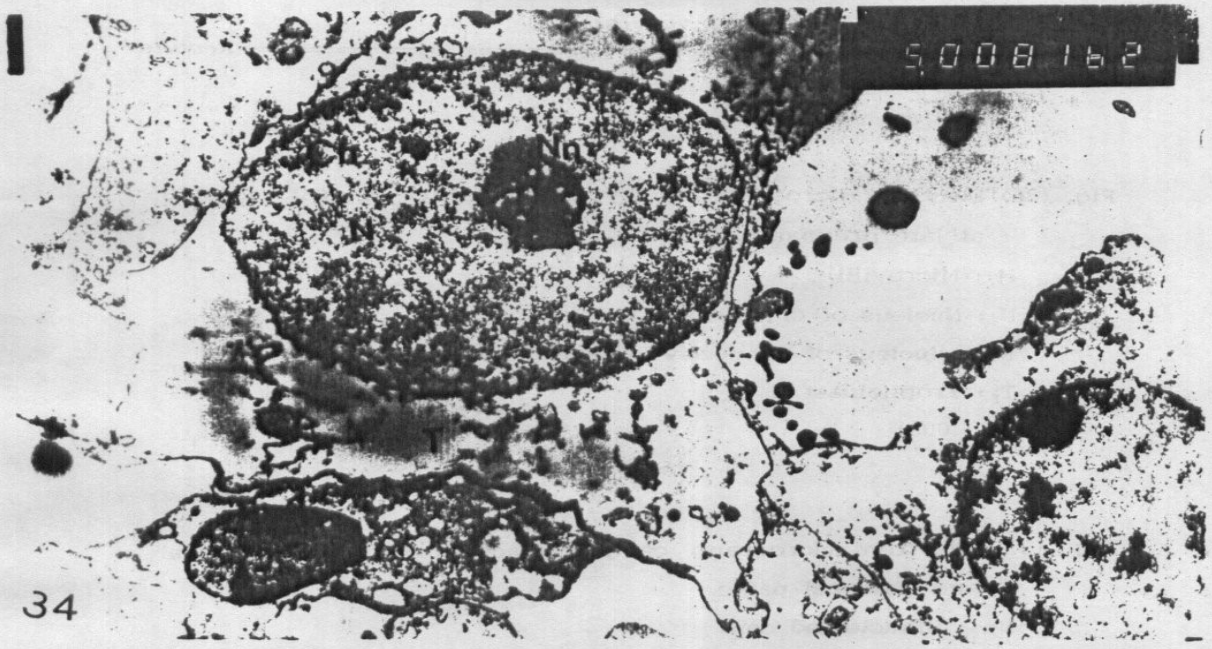
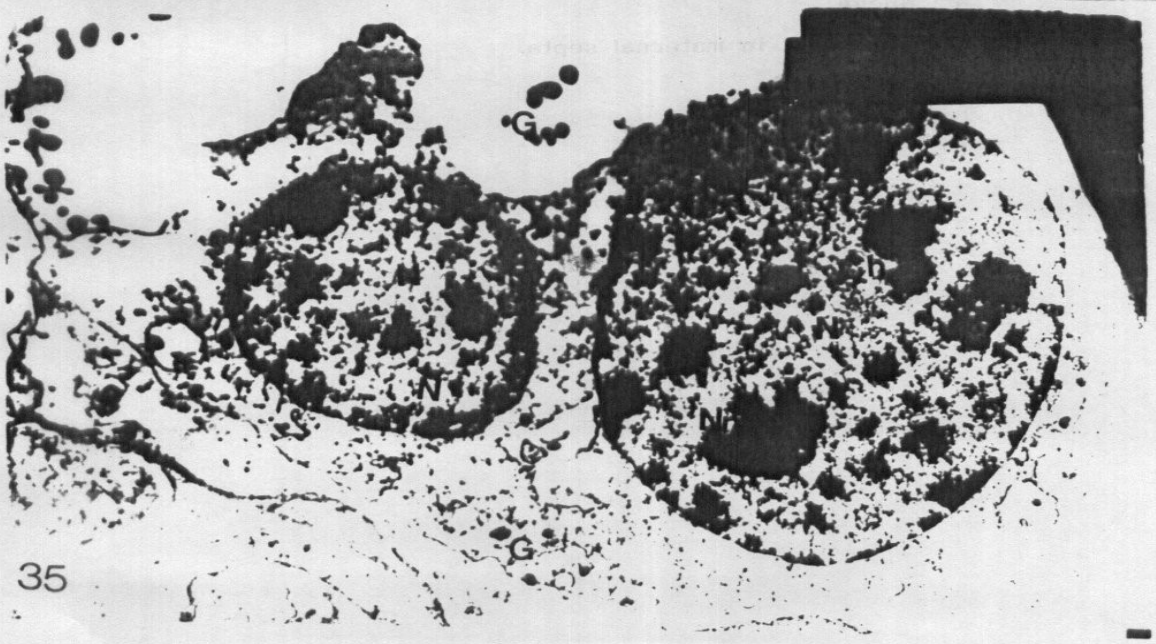

308164
Nf
Tr
N
M
CS
CS
33

Fig. (34) Electron micrograph of the cuboidal trophoblast (T), notice its large nucleus (N) with fine distributed chromatine (Ch) and distinct nucleolus (Nn) and empty cytoplasm (X 5.000).

Fig. (35) Electron micrograph of binucleated giant cell at late pregnancy, Notice its unequal size round nuclei (N) the distribution of their chromatine (Ch), nucleolus (Nu) and the granular cytoplasm (G) (X 5.000).



34



35

Fig. (36) Electron micrograph of cryptal syncytium of goat placenta at late pregnancy showing :

M : Microvilli.

N : Nucleus of cryptal syncytium.

Nc : Nucleus of connective tissue cells.

T_r : Trophoblast.

(X 5.000)

Fig. (37) Electron micrograph of goat placenta showing the fetal and maternal parts.

Bn : Binucleated cell.

Tr : trophoblast.

Msy : Maternal syncytium.

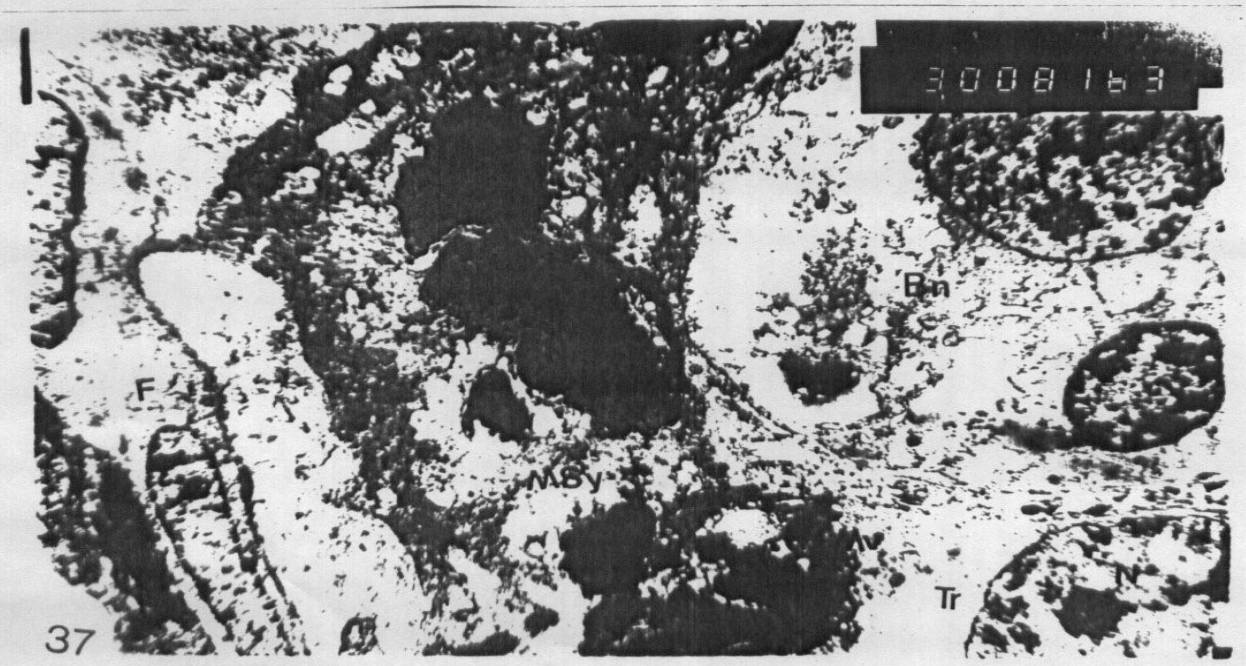
N : Nuclei.

F : Fibroblast in maternal septa.

Mv : Microvilli

(X 3.000)

36



37

Fig. (38) Electron micrograph of the goat placenta showing the structure of the maternal septa.

F : Fibroblasts.

N : Nucleus of the fibroblast

Fa : Fat globules.

Mv : Microvilli.

(X 5.000).

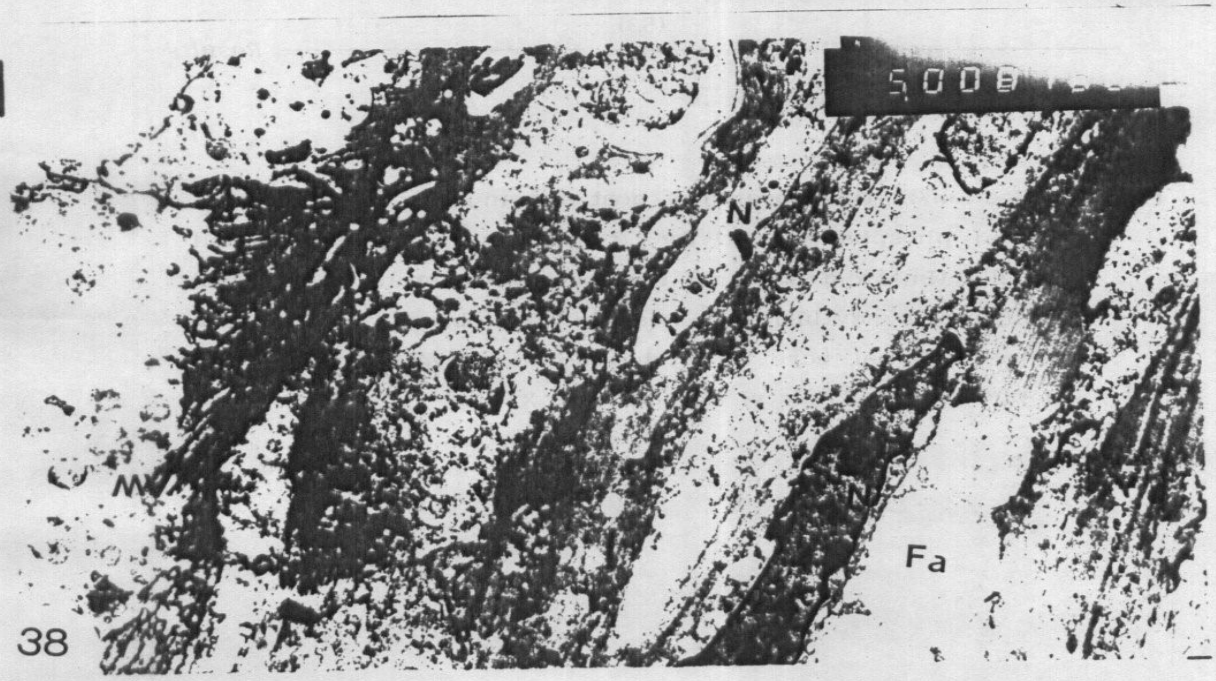
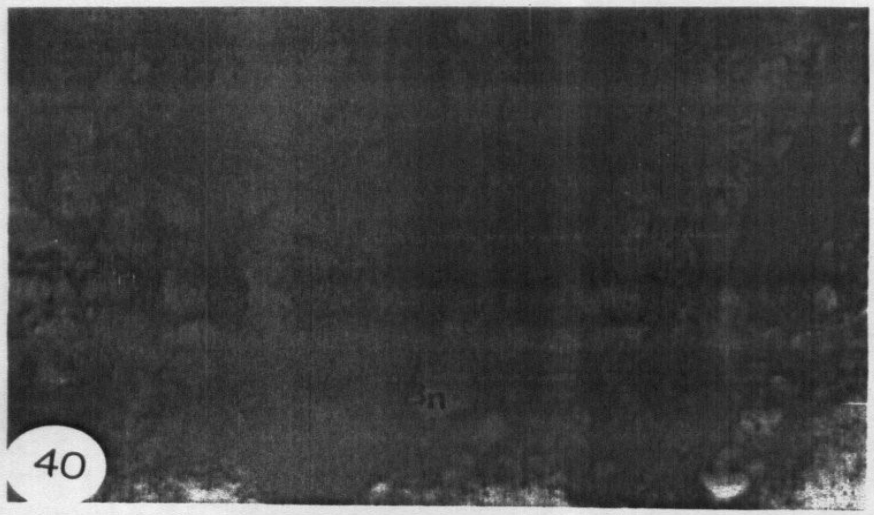

N
Fa
38

Fig. (39) Section in the placenta of goat showing the distribution of acid phosphatase activity. It is high in the binucleated cells (Bn) and maternal septa (Ms), moderate in maternal cryptal syncytium (Cs), weak in trophoblast (T), while the mesenchymal core (Mc) showing negative reaction (Acid phosphatase, Malatyl method, Obj. X 40).


mc
39

Fig. (40) Section in the placenta of goat showing the distribution of alkaline phosphatase activity, it is intense in cryptal syncytium (Cs), moderate in Trophoblast (T), while the binucleated cell (Bn) showing no activity (Alkaline phosphatase, Malatyl method, Obj. X 40).

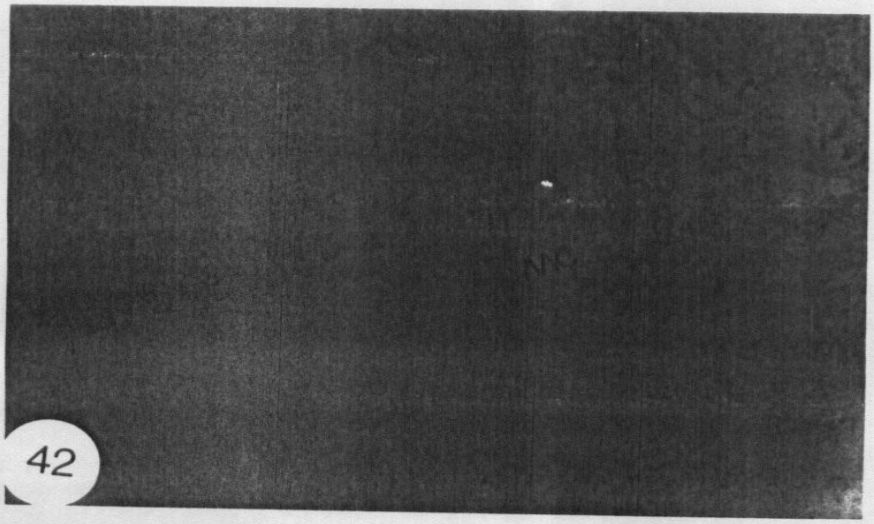
Fig. (41) Section in the placenta of goat showing the distribution of alkaline phosphatase activity in the septal blood vessel (Bl) while the binucleated cell (Bn) showing no reaction. (Alkaline phosphatase Malatyl method, Obj. X 40).



40

41

Fig. (42) Section in placenta of goat showing the distribution of the succinate dehydrogenase activity, it is high in binucleated cell (Bn), moderate in Trophoblast (T) and cryptal syncytium (Cs), while the mesenchymal core (Mc) showing no reaction (Succinate dehydrogenase Lojda method, Obj. X 40)


42


DISCUSSION

DISCUSSION

Regarding the changes that occurred in the caruncular (uterine) epithelium during the attachment and implantation of the balstocyst, there were several suggestion; the first one considered that the uterine epithelium disappeared and the fetal trophoectoderm was apposed directly to the maternal connective tissue ***(Assheton, 1906; Wimsatt, 1951, 1952 and Amoroso, 1952, 1961).*** The ultrastructural study by ***Bjorkman and Blooom (1957)*** in cow and ***Davies and Wimsatt (1966)*** in ewe claimed that the uterine epithelium is lost early at implantation and the trophoectodermal giant cells form the fetal covering of this eroded epithelium giving rise the primitive trophoblastic syncytium and therefore the ruminant placenta classified as syndesmochorial placenta. The second oponion, demonstrated that the uterine epithelium persisted, not destructed at any stage of pregnancy as reported by ***Bjorkman (1965)*** in sheep, ***Lawn et al. (1969)*** in sheep and goat, ***Dent (1973)*** in the goat and ***Steven (1975), Ramsey (1982)*** in cow, consequently, the ruminant placenta was reclassified as epitheliochorial placenta. Our results revealed that the uterine epithelium persist but it was modified to variable degree into a hybrid fetomaternal syncytium formed by the migration and fusion of the fetal binucleated cells with those of the uterine epithelium.

Thus the mature goat placenta is neither entirely syndesmochorial with no uterine epithelium, nor epitheliochorial with two apposed cell layers whose only anatomical interaction is interdigitated microvilli. So, a term which accommodates the variety and recalls the origin of the goat placenta is syn-epitheliochorial type. Similar results was reported by ***Wooding (1992)*** in ruminants.

As reported by previous authors ***Amoroso (1952), Wimsatt (1952), Davies and Wimsatt (1966), Lawn et al. (1969) and Boshier and Holloway (1976 and 1977)*** the present study provides a clear evidence that the villi in goat placenta composed of connective tissue core covered by cuboidal trophoblasts and binucleate cells in between.

In contrast to ***Amoroso (1952)*** who reported that the trophoblasts at the base of the villous is very tall columnar cell, our findings revealed that the base of the villous lined by irregular stratified cuboidal cell layer and the binucleate cells were distributed in different levels in between the cuboidal cells.

The present study revealed that the distribution of the binucleate cells varies along the villous epithelium, it was numerous in the tip and the base of the villi and moderately along the sides of the villous. Similar findings were recorded by ***Wimsatt (1951), Davies***

and Wimsatt (1966) and Wooding (1984) and Lee et al. (1985) in sheep, and *Wooding and Wathes (1980)* in cow.

The migration of the binucleate cells from the fetal to the maternal side of the placentome has been reported by several authors *Amoroso (1952), Davies and Wimsatt (1966), Boshier (1969) and Wooding (1984)* in sheep and *Wooding (1983)* in goat and cow. In the present study the migration of the binucleate cells was observed in all stages of placental development. *Wooding (1983)* added that, this migration appears to serve at least two functions : The transfers of the characteristic granules of the fetal binucleate cells to the maternal circulation, and formation of the placental syncytium bounding maternal connective tissue in goat placentome.

There were two classes of binucleate cells in the goat and sheep placentome has been reported by some authors. On the base of electron microscopic studies, *Boshier and Holloway (1977)* indicated that one type of the binucleate cells was responsible for the synthesis of glycoprotein secretory material and the other type was concerned with glycogen storage. Using immunoflurescence techniques, *Walkins and Reddy (1980)*; and using immunocytochemical studies *Lee et al. (1986); Wango et al. (1991) and Wango et al. (1992)* reported that one class of binucleate cells was associated with production of ovine

placental lactogen and the other with the synthesis of another class of biological active protein or steroid. In the present study, it has been shown that in all stages of placental development, the binucleate cells showed strongly positive PAS granules in its cytoplasm these granules showed some polarity and other binucleate cells indicated degenerative changes with the absent of the PAS positive granules. This finding agree with many authors, who studied the binucleate cells histochemically ***(Wimsatt, 1951)*** in sheep and ***(Bjorkman, 1954)*** in cow. On the other hand, ***Greenstein et al. (1958)*** stated that glycogen was not an important histochemical feature of the developing placenta and diastase resistant PAS positive material indication of carbohydrate protein complex was concentrated in giant cell cytoplasm.

The mechanism involved in binucleate cells origin and development is not completely understood. Several theories have been put forward to explain the origin of the binucleate cells. ***Wimsatt (1951), Lawn et al. (1969), Bosheir and Holloway (1977), Wooding et al. (1980), Wooding (1980, 1982) and Wooding et al. (1986)*** suggested that the trophoblastic origin of the binucleated cell by direct transformation of the ordinary columnar or cuboidal trophoblastic cells, after consecutive nuclear divisions without cytokinoss produce a young binucleate cells. However, ***Greenstein et al. (1958)*** reported that the origin of the bovine binucleate cells from the undifferentiated

(stem cells) which give rise the trophoblastic columnar cells and the binucleate cells. In our study, the evidence of the binucleate cells origin still not fully identified.

In agreement with the results obtained by ***Wooding et al. (1980), Wooding et al. (1981), Wooding (1984, 1992),*** the cryptal epithelium formed by transformation of the uterine (caruncular) epithelium just with contact of trophoectodermal epithelium and migration of binucleated cells and their fusion with individual uterine epithelial cells producing trinucleated fetomaternal hybrid cells, and with continued migration of the binucleate cells, fusion, death or replacement of the remaining uterine epithelial cells occur resulting to the formation of syncytial plaque. On the other hand, ***Ludwing (1962) and Lawn et al. (1963, 1969)*** suggested that cryptal epithelium might be formed by modified uterine epithelium.

There are two opinions concerning the origin of the cryptal epithelium layer. It may be fetal in origin as reported by ***Assheton (1906), Amoroso (1952)*** this opinion supported recently by electron microscope study by ***Wooding (1980)*** and autoradigraphy study by ***Wooding et al. (1981).*** It may be also formed as a result of the fusion of original uterine epithelial cells as reported by ***Ludwing (1962), Bjorkman (1965), Lawn et al. (1969), Boshier and Holloway (1977) and***

Steven et al. (1978) in sheep and goat, ***Bjorkman and Bloom (1957)*** in cow and ***Dempsey et al. (1955)*** in pig. This oponion was supported recently by ***Chamber (1978).***

Our finding revealed that the nature of the goat cryptal epithelium was syncytial less cellular. This is in agreement with the results of ***Amoroso (1952), Bjorkman (1954 and 1969)*** and ***Ludwing (1962)*** who indicated that marked variation from one species of ruminant to another, and the cryptal epithelium is mainly cellular in cow, syncytial in sheep, however, ***Lawn et al. (1969)*** indicated cellular nature of the cryptal epithelium in sheep and goat placentome.

In our study, the binucleate cells were directly involved in the modification of the uterine epithelium into syncytial plaque, this modification began early at implantation and continuous until term. These in agreement with the result of ***Wooding (1982, 1983) and Wooding et al. (1986)*** in different ruminant placenta. Thus in the goat and sheep all the placentomal membranes originated from the fetomaternal syncytium at the fetomaternal interface as stated by ***Wooding (1984)*** and ***Wango et al. (1990 a, b)***, while in the cow and deer, although at implantation, the caruncles were covered by fetomaternally derived syncytium, this was displaced by regrowing of the unicellular uterine epithelium and binucleated cells migration and

fusion produced only isolated transient uterine trinucleated cells which soon die ***(Wathe and Wooding, 1980; Wooding and Wathes, 1980).***

In agreement with ***Wooding (1984)***, the fetomaternal syncytial plaques provided an essential immunological and biological barrier, as they form only a part of the fetomaternal interface and lack the continuity of a true syncytium, which has no lateral boundaries. This reduction of the diffusion distance between the maternal and fetal blood capillaries is achieved in several different way in different placental types.

The observation that intracellular materials in the binucleate cells were PAS positive suggested that these cells can synthesis and store glycoprotein. Certainly, the association between the presence of granular endoplasmic reticulum, membranous bound storage granules is suggestion of the secreting role for some of binucleate cells.

Placenta is an organ servicing psychological exchange between the mother and fetus sides ***(Mossman, 1937)***, these exchanges may be done by diffusion, active transport or by pinocytosis ***(Moya and Thorndike, 1962)***. The microvillous junction between the syncytium and trophoblast that observed in the present study suggests to facilitate this function.

The occurrence of secretory granules in some of trophoblastic cuboidal cells support the suggestion of ***Djione and Kann (1975) and Perry et al. (1975)*** that the ovine serum contain Ovine Placental Lactogen (OPL) at the late stage of pregnancy. These results required further study to investigate these granules with immunohistochemical technique using unlabeled antibody.

In some uninucleated trophoblast cells of full term placenta, short branching tubules of agranular endoplasmic reticulum were distinct, this indicated that these cells were concerned with the production of steroid hormones ***(Fawcett et al., 1969).***

Widespread degenerative changes of binucleate cells were observed mainly in the tip of the chorionic villi during the gestation period of goat. Similar observation was reported by ***Bjorkman and Sollen (1961) and Bjorkman (1969)*** in bovine placenta. This may be autolytic processes necessary for the continuous growth and remodeling of the developing placentome.

Ultrastructurally, there was a microvillous interdigitation between trophoblasts and syncytium in the goat placenta, a result which agreed with that described by ***Hamilton et al. (1960)*** in

cervidae, *Ludwing (1962, Bjorkman (1965)* and *Lawn et al. (1969)* in sheep and goat, *Bjorkman and Bloom (1957)* in cow.

The determination of enzyme levels in the cells represents one approach towards the understanding of the metabolism and mechanism by which cellular changes are affected, although such information is quite limited because the histochemical demonstration of enzymes does not permit definite conclusions concerning the actual state of metabolism *(Edress et al., 1988).*

The obtained data in the present study demonstrated the presence of acid and alkaline phosphatase as well as succinate dehydrogenase in the villous and cryptal epithelium of the placentome of goat. Since the role of alkaline phosphatase in the adjustment of the permeability of the cell membrane and the exchange of substances through it has already been reported by *(Molbert et al., 1960 and Hashimoto and Ogawa, 1963),* the presence of the this enzyme in the villous and cryptal epithelium in high amount indicates that this cells are concerned with material exchange and metabolism between maternal and fetal sides.

In agreement with our finding, *Fahmy (1957), Christic (1967, 1968), Mourdoch (1970), Zamiri (1980) and Roy and Saigal (1987)* in

sheep, observed that intense granular alkaline phosphatase activity in the trophoblastic cells and moderate to strong activity in the cryptal cells. ***Bosheir (1969)*** concluded that the presence of alkaline phosphatase activity at the junction of the trophoblast and maternal epithelium has been associated with carbohydrate metabolism and the production of trophoblastic fibrinoid.

High alkaline phosphatase activity was observed in the wall of the capillaries of the maternal septa coinciding with the earlier finding by ***(Bosheir, 1969), Mourdoch (1970) and Roy and Saigal (1987)*** in sheep. On the other hand, the weak activity of alkaline phosphatase in the chorionic villi and its mesenchymal cells was reported also by ***Fahmy (1957), Christic (1967, 1968)*** and ***Roy and Saigal (1987)*** in sheep placenta.

The present finding revealed that the binucleated cells lack alkaline phosphatase reaction, in contrast to this result, ***Amoroso (1952), Weeth (1953) and Foley et al. (1954)*** who reported intense alkaline phosphatase in the binucleate cells in bovine placenta.

Acid phosphatase which is generally associated with catabolic processes ***(Hashimoto and Ogawa, 1963 and Ellis, 1964)***, may be involved in destruction, phagocytosis and digestion of the dead cells

as a result of renewal and remodeling of the placentome. As well, acid phosphatase is important in the energy transfer through high energy phosphate compounds. Accordingly, the existence of positive reaction for this enzyme would be likely in an active secreting glands or cells ***(Wight et al., 1971).***

The marked increase in acid phosphatase activity in the binucleate cells that observed in the present study is in agreement with the suggestion that acid phosphatase release has been associated with lysosomal involvement in implantation and placentation in sheep ***(Boshier, 1969)***, in man ***(Contractor et al., 1977)*** and in mouse ***(Moulton et al., 1078).*** On the other side, weak to moderate activity of acid phosphatase was observed in trophoblast cells is in agreement with the observation of ***Sharma et al. (1983)*** in buffalo and ***Roy and Saigal (1987)*** in sheep.

Succinate dehydrogenase which is frequently used as a marker for the activity of the citric acid cycle has been found in greatest concentrations within the villous epithelium indicate the high metabolic activity in these cells ***(Formison and Montagna, 1954 and 1955).***

Our finding of intense succinate dehydrogenase activity in the cryptal cells and binucleate cells supported by the finding of ***Roy and***

Saigal (1987) in sheep. While succinate dehydrogenase activity was moderate in the trophoblast cells in the goat placenta it was intense in the cattle as reported by ***Friess and DeBaries (1971)*** and in sheep by ***Roy and Saigal (1987).***

The binucleate cells and maternal septa showed a strong reaction with PAS, and moderate to week reaction was observed in the connective tissue core of the villi. Similar observations were described by ***Wimsatt (1951), Boshier (1969), Datta et al. (1979) and Kosaric et al. (1986)*** in sheep. In bovine placenta, ***Wimsatt (1951), Bjorkman (1954) and Greenstein et al. (1958)*** reported that the binucleate cells were found to be glycogen free and the PAS positivity resulting from intracytoplasmic concentration of carbohydrate-protein complex. The glycogen represents a ready source of energy, and is possibly a substance that can be used as intermediary product for the synthesis of other substances in the cell ***(Montagna et al., 1951).*** Thus, the presence of glycogen in the binucleated cells and maternal septa might represent the source or one of the sources of energy for the synthesis of proteins in these cells and migration of the binucleated cells.

In agreement with the observation of ***Bhattachary and Saigal (1984) and Roy and Saigal (1986),*** the trophoblast cells showed a

moderate PAS positive reaction while maternal septa give strong reaction.

In our finding, alcianophilic reaction of acid mucosubstances was found in the mesenchymal portion of the placenta, this confirm the observation of ***Kozaric et al. (1986)*** in sheep placenta. While weak alcian blue was observed in the chorionic epithelium including trophoblast and binucleate cells and in cryptal syncytium as also reported earlier in sheep placenta by Roy and ***Saigal (1986)*** and in binucleate cells of cow placenta as reported by ***Wimsatt (1951)***.


SUMMARY

SUMMARY

The present study was carried out to illustrate the morphological and histochemical structure of the placenta of the native (Baladi) goat at different stage of gestation period.

The results revealed that, the doe's placenta is formed of two main components, The placentome and intercotyledonary area. The placentomes were button shaped and arranged in four rows, two dorsally and two ventrally. The placentome formed of fetal side (cotyledone or chorionic villi) and maternal side (caruncle).

Histologically, the cotyledon formed mainly of the chorionic villi, these villi were branched and fastened in the maternal crypts found in the caruncle. The lining epithelium of the chorionic villi or trophoectoderm formed of two cell types, cuboidal or polyhedral cells with slightly acidophilic cytoplasm and central located large nucleus, and binucleated, strongly acidophilic cell scattered in between the former cells. The binucleated cells were in two forms, one with strongly PAS positive reactivity indicating more carbohydrate storage,

the other with less PAS positive and more cell organelles indicating steroid hormone secretion.

The mesenchymal connective tissue core showed weak PAS reaction but it was strongly alcianophilic. Its predominant cell components were undifferentiated mesenchymal cells and fibroblasts. With the advancement of the pregnancy, there were wide blood spaces underling the basement membrane of the trophoectoderm decreasing the distances between maternal and fetal blood.

The cryptal lining epithelium was formed as a result of migration of the binucleated cells to the uterine epithelium and made fusion with it resulting to a hybrid fetomaternal syncytium. This indicating that the goat placenta neither syndesmochorial nor epitheliochorial, but fetomaternal interference of syn-epitheliochorial type.

The cryptal syncytium and maternal septa showed strongly PAS positive reaction and weak to moderate alcianophilic reaction. The maternal septa was formed of fibrous and cellular elements. The fibrous elements was mainly collagen while the cellular element formed of fibroblast like cells.

Microvillous interdigitation between the trophoblast cuboidal cells and syncytium was observed in the placenta of goat.

Strong acid phosphatase activity was observed in the binucleate cells and cryptal syncytium, moderately to strong in maternal septa, and moderate to weak in the trophoblast.

Alkaline phosphatase was localized mainly in the trophoblast cuboidal cells and in the cryptal cells.

Succinate dehydrogenase was observed nearly in all cell population of the placentome.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ARABIC SUMMARY

الملخص العربى

- بعض الدراسات المورفولوجيه والنسجوكيميائيه على مشيمة الماعز

تم إجراء هذا البحث على مشيمة الماعز (البلدى) لتوضيح التركيب المورفولوجى والنسيجى للمشيمه فى مراحل الحمل المختلفه .

وقد أظهرت الدراسه النتائج الأتيه :

- أن مشيمة الماعز تتكون من مكونين رئيسيين هما الفلقات (القرص المشيمى) والمسافات بين الفلقيه . والقرص المشيمى فى الماعز يأخذ الشكل القرصى (الزرار) وتترتب فى أربع صفوف زوجين من الصفوف الظهريه وزوجين من الصفوف فى الناحيه البطنيه للرحم . بحيث يتكون القرص المشيمى من إتحاد حملات المشيمه الخارجيه مع بروزات جدار الرحم .

- أظهرت الدراسه النسيجيه أن الخملات الخارجيه للمشيمه تتفرع وتثبت نفسها بالأخاديد الناتجه ببروز جدار الرحم .
- إن الأغشيه الطلائيه المبطنه للخملات تتكون من نوعين من الخلايا . الخلايا الغازيه المكعبه والتى تحتوى على نواه مستديره مركزيه وسيتوبلازم قليل الحامضيه . والخلايا العملاقه ذات النواتين التى تحتوى على سيتوبلازم شديد الحامضيه وتوجد هذه الخلايا بقله وموزعه بين الخلايا الغازيه المكعبه .

- الخلايا الغازيه العملاقه ذات النواتين يوجد منها نوعين الأول يتفاعل بشده مع PAS مما يدل على وجود كميات من المواد الكربو هيدراتيه المخزنه داخل السيتوبلازم . والنوع الأخر منها يظهر تفاعل محدود مع ال PAS ولكنها تحتوى على الشبكه

الأندوبلازميه وعلى عدد كبير من العضيات الخلويه مما يؤكد قدرتها على إفراز هرمونات الاستيرويدات .

- الأغشيه المسراقيه التى تملأ تجويف الخملات تتفاعل تفاعل محدود بال PAS ولكنها تتفاعل بشده مع Alcine الأزرق وقد وجد أن الخلاياالسائده هى الخلايا الليفيه والخلايا المسراقيه غير المميزه .

- أظهرت الدراسه النسيجيه أيضا أن الأوعيه الدمويه السحقيه الموجوده بالخملات الجنينيه تزداد مع تقدم مراحل الحمل وتتركز أسفل الجدار السفلى للخلايا الغازيه المكعبه ليقلل من الحاجز المشيمى بين دم الأم ودم الجنين .

- الأغشيه المبطنه للأخاديد الموجوده بالقرص المشيمى تتكون من هجرة الخلايا الغازيه العملاقه ذات النواتين وإندماجها مع الخلايا المبطنه لجدار الرحم عند مراحل الحمل الأولى وتتحول إلى خلايا مخلويه مسطحه أو مكعبه . هذه الخلايا تتفاعل بشده مع PAS

- أظهرت الدراسه أن الطبقه المخلويه والفاصل الأخدودى يتفاعلان بشده مع PAS ولكنها تتفاعل تفاعل ضعيف إلى متوسط مع Alcine الأزرق ويتكون الفاصل الأخدودى من الياف ال Cllagen ومن الخلايا الليفيه .

* أظهرت الدراسه بإستخدام الميكروسكوب الالكترونى النافذ وجود خملات دقيقه متداخله بين الخلايا الغازيه المكعبه وبين الطبقه المخلويه فى مشيمة الماعز .

*** أظهرت الدراسه النسجوكيميائيه الأتى :**

أن إنزيم الفوسفاتيز الحامضى يتفاعل بشده مع الخلايا الغازيه العملاقه ذات النواتين

وكذلك مع الطبقه المخلويه ويقل التفاعل إلى متوسط مع الفاصل الأخدودى ويندر مع الخلايا الغازيه المكعبه .

-إن إنزيم الفوسفاتيز القاعدى يتركز فى الخلايا الغازيه المكعبه والطبقه المخلويه .

- وجود إنزيم السكسينات Succinate تقريبا فى كل خلايا المشيمه بكميات محدوده

بسم الله الرحمن الرحيم

والأنعام خلقها لكم فيها دفء ومنافع ومنها تأكلون

﴿صدق الله العظيم﴾ سورة النحل الآيه ٥

بسم الله الرحمن الرحيم

نبذه عن تاريخ حياة الباحث

الإســـــم | أمـــين محمـــد أحمـــد حســـانين

تاريخ الميلاد| ١٣-١١-١٩٦٦

محل الميـلاد | نجع مازن غرب – البلينا – سوهاج

حصـــل الباحث على الشهاده الإبتدائيه سنة ١٩٧٨ من مدرسة نجع مازن غرب الإبتدائيه

حصـــل الباحث على الشهاده الإعداديه سنة ١٩٨١ من مدرسة الشيخ مرزوق الإعداديه

حصـــل الباحث على الشهاده الثانويه سنة ١٩٨٤ من مدرسة البلينا الثانويه المشتركه

إلتحـــق بكلية الطب البيطرى جامعة أســـيوط سنة ١٩٨٤

حصـــل على بكالوريوس العلوم الطبيه البيطريه –دور مايو سنة ١٩٨٩ بتقدير عام جيد جداً مع مرتبة الشـــــــرف

عين معيـــــداً بقســـم التشريح والفسيولوجيا – كلية الطب البيطرى – بكفر الشـــيخ جـــامعـــــة طنطـــــا بتاريخ ٢-٦-١٩٩١

مســجل لدرجة الماجســـتير فى مادة الفسيولوجيا فى سبتمبر ١٩٩٢

١٨٩

بسم الله الرحمن الرحيم

بعض الدراسات المورفولوجيه والنسجوكيميائيه على مشيمة الماعز

رسالة مقدمة من

ط.ب./ أمين محمد أحمد حسانين

للحصول علي درجة الماجستير في العلوم الطبية البيطرية
(هستولوجيا)

تحت إشراف

الإستاذ الدكتور/ على عبد القادر منصور
أستاذ التشريح والاجنة
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