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A. Y. Yasear
Department of Dentistry, Hilla University College, Babylon, Iraq, akram.yy@hilla.unc.edu.iq

A. H. AL-Saffar
Department of Dentistry, Hilla University College, Babylon, Iraq, aahalsaffar@yahoo.com

R. E. Moore
Department of Dentistry, Hilla University College, Babylon, Iraq, rafad.emad@hilla-unc.edu.iq

E. R. Arbuthnott
Department of Dentistry, Hilla University College, Babylon, Iraq

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An Ultrastructural Study on the Melanocytes of the Pregnant ewe Uterus

A. Y. Yasear, A. H. AL-Saffar, R. E. Moore, E. R. Arbuthnott

Department of Dentistry, Hilla University College, Babylon, Iraq

Abstract

The basic feature of melanocytes of ewe uterus is similar to those present in many places in the body. They all have the ability of melanin production and the origin from neural crest cells.

Samples from thirteen ewes were used in this study at eight different stages of pregnancy. They processed for examination under light microscope and electron microscope.

The caruncular endometrial tissue at day 16 of pregnancy contains large number of melanocytes. These cells changes as the foetal villi advance into the endometrium. At day 29 of pregnancy the melanocytes granules were beginning to be engulfed by lysosomes. By day 66 of pregnancy the pigment granules have virtually vanished from the endometrium. By day 75 all stromal cells have disappeared. We suggest that a process autophagocytosis was the reason behind the disappearance of the melanocytes. The function of melanocytes and their disappearance during pregnancy in the ewe is still intriguing.

Keywords: Melanocytes, ewe uterus

1. Introduction

Melanocytes are cells of neural crest origin [1]. The favorite habitat of melanocytes is the epidermis of skin [2]. Melanocytes have also been found in in the inner ear, nervous system, and heart [1, 3], and in the gingiva of oral mucosa [4].

The endometrium of ewe is modified to have from 60 to 90 circumscribed raised area called carnucles [5, 6].

During pregnancy the carnucles are considered as the maternal part of placenta in the sheep and other ruminants which receive the foetal parts called cotyledons, where form together the placentomes [5, 6].

There are two characteristic populations of stromal cells types present in the carnucles; the stromal cell proper and the melanocyte [6, 7]. The latter is structurally similar to the melanocyte found in the skin and elsewhere in the body and can be found in the uterus of the non-pregnant ewe and even in the uteri of foetal lambs [8]. The black pigmentation in the uterine caruncular mucosa is due to melanocytes [5, 9–11] and not haemtogenous granules as suggested by Bonnet [12] or Pseudomelanin [13]. By the 42nd day of pregnancy few if any of the original population of the melanocytes remain [7, 14]. In an earlier paper the former authors [15] had demonstrated that all melanocytes together with most of maternal connective tissue had been replaced by many layers of basement membrane-like material at day 78 (dpc) the earliest stage studied. The pigmentation of the uterine caruncle is not fully restored until at least 14 days after parturition [14]. The function of melanocytes in the reproductive tract is unknown and there are few descriptions of their morphology and fate. Due to the lack of adequate information on the melanocytes of the pregnant ewe uterus, this paper was aimed to shed more light on the histology of the melanocytes in the caruncular endometrium of the ewe throughout pregnancy.

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E-mail addresses: akram.yy@hilla.unc.edu.iq (A. Y. Yasear), aahalsaffar@yahoo.com (A. H. AL-Saffar), rafad.emad@hilla-unc.edu.iq (R. E. Moore).

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2. Materials and methods

Uteri from thirteen ewes were used in this study at eight different stages of pregnancy. Nine were dated pregnancies viz: day 17 of pregnancy(day post coitum = dpc); number of ewes(n) = 2; day 29 (dpc) n = 2; day 35 (dpc) n = 1; day 47 (dpc) n = 1; day 66 (dpc) n = 2 and day 75 (dpc) n = 1. In addition, four uteri were obtained from local slaughter house, pregnancy dating was estimated from fetal crown rump length [17] viz: days 35, 47, 70, 120 (dpc). The thirteen uteri were removed within 10 minutes of death, the middle uterine artery of each uterus was cannulated and the organ was flushed with about 100 mL w/v NaCl saline solution.

This was followed by a Karnovsky fixative [16] which is consisting of 2% paraformaldehyde: 2% gluteraldehyde solution in 0.1 M phosphate buffer pH 7.2. The uteri were slowly perfused via the middle uterine artery for about 20 minutes. Match-stick sized pieces were then excised by cutting vertically down from the surface of several placentomes (the placentome represent a localized areas in ewe uterus in which the fetal chorionic villi are interlocked with maternal tissue). The collected samples were further immersed fixed in the same fixative for 60–90 minutes at 4 °C. Post fixation and embedding: All the materials were washed in several changes of cold (4C) phosphate buffer, postfixed in 2% osmium tetraoxide in 0.1 M phosphate buffer pH 7.2 for 2 hours at room temperature, dehydrated in ascending grades of alcohol, cleared in propylene oxide and embedded in epon araldite mixture. One micron serial sections were cut using an LKB III ultratome and stained with 1% toludine blue for light microscopy. For electron microscopy, 40–70 nm thin sections were collected on uncoated grids and stained with uranyl acetate and lead citrate. They were examined using a Hitachi HU12A electron microscope at 75 Kv.

3. Results

At days 17, 29, 35 and 47 (dpc), melanocytes were seen distributed among other stromal cells of the endometrial caruncular tissue which were fibroblast-like cells (Fig. 1). Their position has no apparent relationship with either the maternal blood vessels or the other stromal cells.

The melanocytes observed at this stage of pregnancy always contain large number of discrete pigment granules as their predominant cytoplasmic components (Fig. 2). These inclusions were found

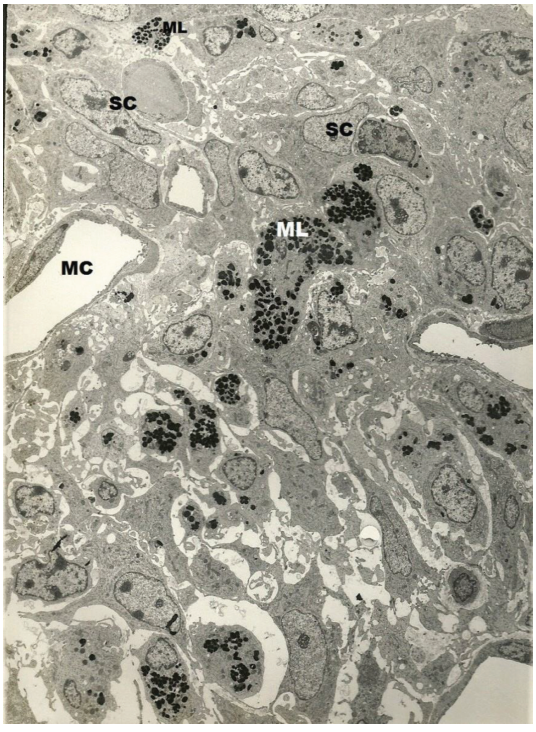

Fig. 1. Low–power electron micrograph in endometrial caruncular tissue of ewe pregnant at day 47 dpc. The melanocytes (ML) with characteristics dark melanin granules are distributed among the stromal cells (SC) and maternal capillaries (MC). X7500.

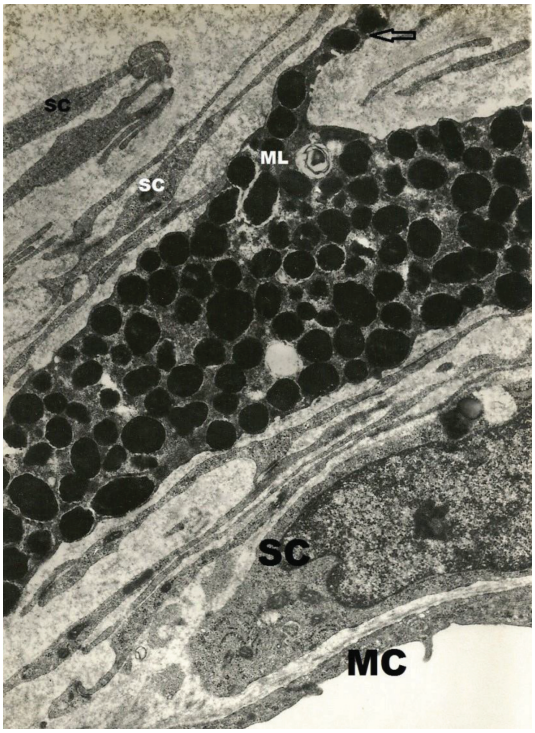

Fig. 2. Day 17 of pregnancy. The micrograph is showing melanocyte (ML) at higher magnification surrounded by stromal cells (SC), and nearby maternal capillary (MC). The melanin granules are the predominant feature of the melanocyte cytoplasm. The granules are also seen in the processes of the melanocyte (arrow) at this stage of pregnancy, other cytoplasmic organelles are obscured by the melanin granules. X 15000.

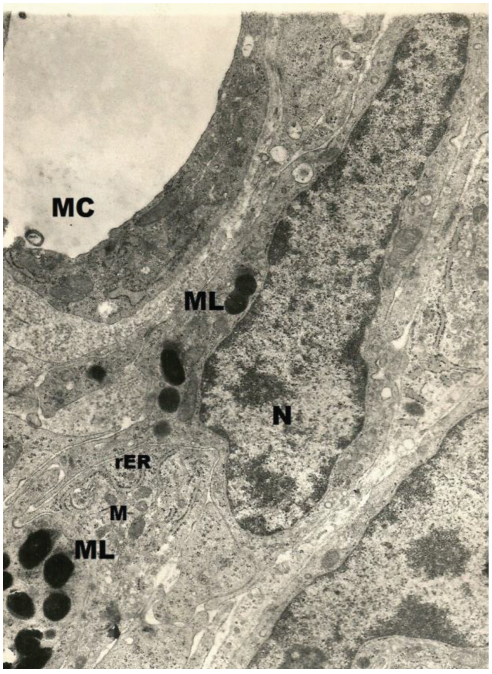

Fig. 3. Day 35 of pregnancy (dpc). The differentiating melanocytes (ML) contain less melanin granules, which appear dark in the micrograph, but other cytoplasmic organelles are more obvious compared with preceding micrograph. Note the abundant rough endoplasmic reticulum(rER) and mitochondria (M), The nucleus (N) of melanocytes possess marginal heterochromatin and central heterochromatin. MC = maternal capillary. X 15000.

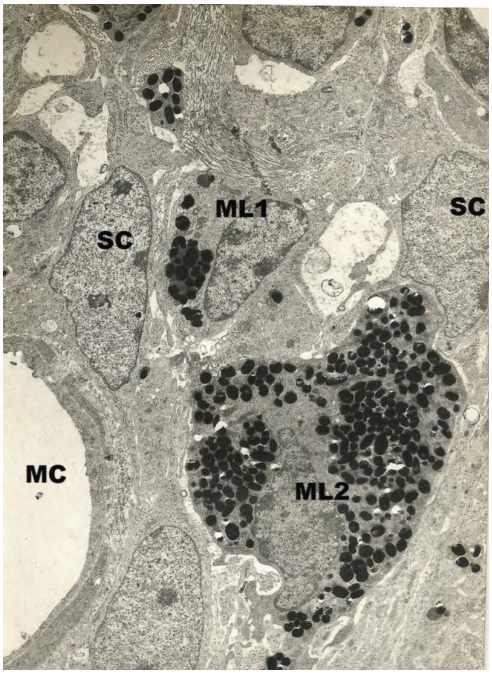

Fig. 4. Micrograph of section from Day 47 of pregnancy (dpc) showing the signs of differentiation in the melanocytes (ML1 and ML2) similar to that of neighboring stromal cells (SC). Note the similarity of the nuclei in both the melanocyte and the other stromal cells. MC = maternal capillary. X 15000.

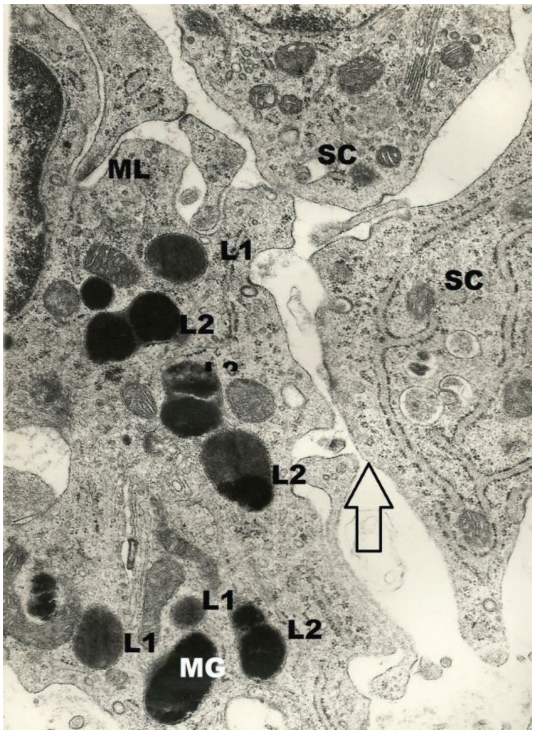

Fig. 5. The micrograph, taken from day 47 pregnant uterus, is showing melanocyte (ML) with appositional contact (arrow) with neighboring stromal cells (SC). The most prominent feature is the appearance of primary lysosomes (L1) which have phagocytized melanin granules and converted into secondary lysosomes (L2). Free melanin granules (MG) are also seen. X 25000.

throughout the cytoplasm of the cells including the perinuclear region and the few short cytoplasmic processes. The nucleus was mostly obscured by the pigment granules. Other cell organelles were scanty.

At days 29 till day 47 the melanocytes have begun to show signs of differentiation and the cytoplasmic organelles become more obvious, compared with melanocytes of day 17.

These include abundant short tubules of rough endoplasmic reticulum and many mitochondria (Figs. 3 and 4). The nucleus has now conspicuous marginal heterochromatin and resembles that of the other stromal cells (Fig. 4). At the same time there were also numbers of primary lysosomes, some of which were in different stages of engulfment of the melanin granules to form secondary lysosomes were encountered in the cytoplasm (Fig. 5). Free melanin granules could be seen in these cells (Figs. 5 and 6). The cell outline was more irregular than at day 17 and the cells appear to be much larger, so that the dark melanin granules no longer obscure other cytoplasmic constituents. The contact between the melanocytes and the neighboring stromal cells was merely appositional and no specialized junction was observed (Figs. 6 and 7).

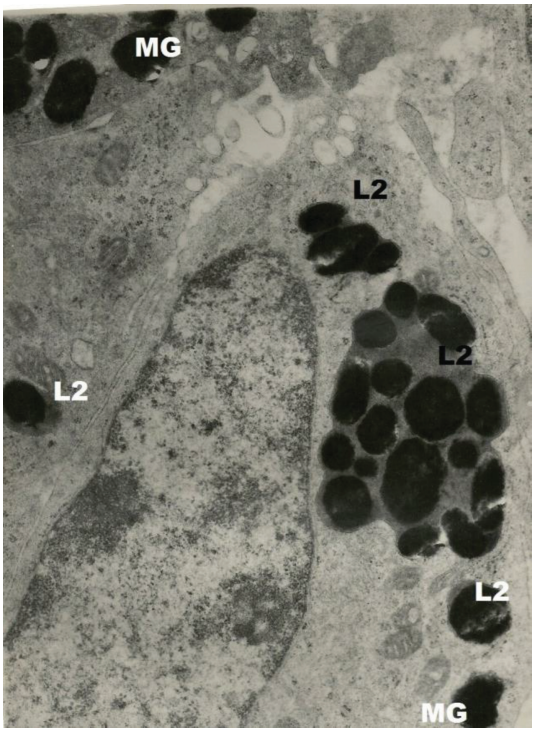

Fig. 6. Day 47 of pregnancy. The micrograph shows part of a differentiating melanocyte with few melanin granules (MG). Some of these granules are already engulfed within secondary lysosomes (L2). Other melanin granules (MG) are free in the cytoplasm. The melanocyte is exhibiting autophagocytosis for the melanin granules. X 27000.

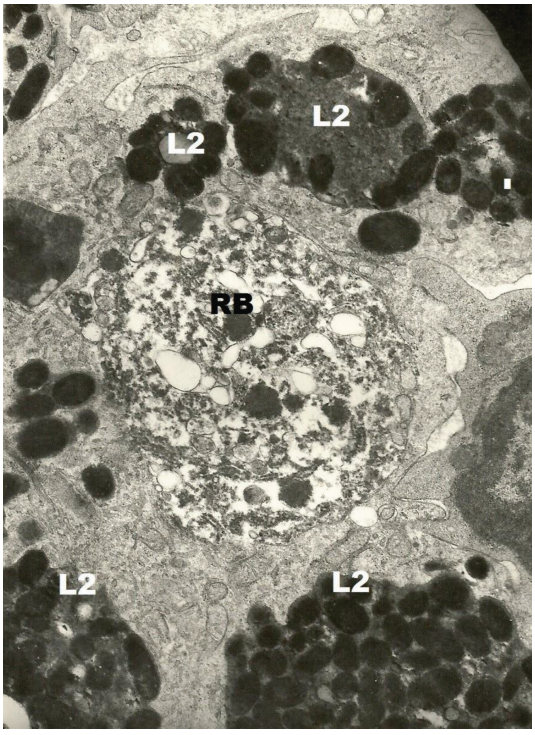

Fig. 7. Micrograph of section taken from pregnant uterus at day 66 (dpc) shows massive process of degradation of the melanin granules by lysosomes which are converted into secondary lysosomes (L2). Some of degraded melanin granules are seen in cytoplasm as residual body (RB). X 27000.

The process of degradation of melanin granules continues until day 66 of pregnancy, some of degraded melanin granules are seen in cytoplasm as residual body (Fig. 7). By day 75 till the end of pregnancy all the melanocytes together with other stromal cells of the maternal side of the placenta have disappeared (Fig. 8) leaving only pericytes intervene between the syncytium and the endothelial cells of the maternal microvasculature.

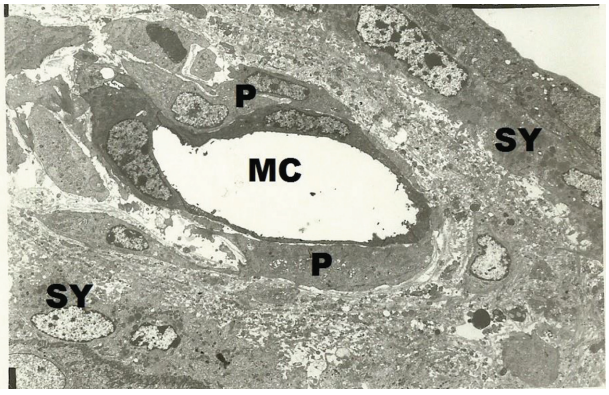

Fig. 8. Micrograph of section from 75 pregnant ewe showing the disappearance of the melanocytes and other stromal cells leaving the maternal capillary (MC) only separated from the syncytium (SY) by the pericytes (P). X 15000.

4. Discussion

The melanocytes were obvious components of the caruncular endometrial tissue at day 17 of pregnancy. Evidence of degradation of melanin granules starts to appear at day 29 and somewhat later. By day 66 melanocytes have virtually disappeared.

The question of the disappearance of the melanocytes poses certain problems. Did they differentiate into other stromal cells or were they destroyed and removed by other cell types. At day 17 of pregnancy there were two clearly distinguishable stromal cell types; one packed with melanin granules and few cytoplasmic organelles and the other containing organelles and no melanin granules. If the melanocytes were destroyed one would expect to find debris in the intercellular spaces, and occasional disintegrating cells. But no sign of these cells have been observed and the melanin granules were such obvious cell components that it was difficult to believe that these would not be seen in some on the hundreds of sections examined. It was possible, nevertheless, that macrophages could engulf melanocytes piecemeal, and not leave

any intercellular debris. This seems unlikely since the secondary lysosomes appear to be packed with melanin granules, with no signs of other cytoplasmic constituents. The alternative hypothesis, autophagocytosis, is therefore more attractive and evidence of this must be sought. Melanin granules in the early melanocytes were free in the cytoplasm as far as can be ascertained, and have no surrounding membrane. In the later stages i.e. day 29 of pregnancy onwards the granules were frequently seen within lysosomes and surrounded by lysosomal membrane. Occasionally both sets of granules i.e. free and engulfed, could be seen within the same cells and this appears to suggest that autophagocytosis, is occurring. Autophagocytosis is an essential process in the melanocytes of the skin to and its survival [18]. Moreover, it has been found that autophagocytosis is a cellular event important for controlling tissue homeostasis and maintaining various normal and pathologic processes in human diseases including cancer [19]. However, the function of the melanocytes in the sheep uterus during pregnancy is still unclear.

The reason for the differentiation and eventual disappearance of the cells is still intriguing. As the foetal advances deeper and deeper into caruncular endometrial tissue there were marked concomitant changes in all tissue of the carnucules [5, 7, 20]. Several excellent studies on the placenta of small ruminants [20–24] have pointed out that the advancement of fetal villi will bring about continuous waves of binucleated trophoblastic cells migrating, throughout pregnancy, from the fetal side of the placenta toward the maternal side of the placenta. Some of the contents of the binucleated trophoblastic cells have been revealed. They found to contain placental lactogen [22–24]. Thus, it would appear that the binucleated cells and their contents were instrumental in these changes. If chemical agents are involved we have no evidence as to its nature and source. However, by day 47 of pregnancy the advancing villi have almost filled the space superficial to the myometrium leaving only maternal blood vessels and their associated pericytes. Thus all stromal cells have effectively disappeared by day 75 of pregnancy in the caruncular region [7], reducing the placental barrier between the foetal and maternal circulation.

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