

HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE DECIDUA AND THE UTERINE GLANDS DURING EARLY PREGNANCY IN THE RAT (*RATTUS RATTUS*)

By

A. M. EL-SHERSHABY, A. E. EL-AGAMY* and S. K. KALDAS

Department of Biology, College of Education King Saud University,

Abha Branch, Saudi Arabia and Department of Zoology

Faculty of Science, Zagazig University, Arab Republic of Egypt

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ABSTRACT

Histological and histochemical studies were carried out on the decidua and uterine glands in the uteri of pregnant rats '*Rattus rattus*' between 129 hours post-coitum (p.c) and day nine of pregnancy. Decidualization was increased from 129 h p.c. to 153 p.c. and reached its maximum at day nine of pregnancy. The decidual cells showed a dense basophilic cytoplasm and large nuclei. Each nucleus contained one to five deeply stained nucleoli. Cells of the decidua appeared rich in proteins, RNA-containing particles and polysaccharides. These biological materials were present in considerable amounts in the intercellular spaces and very near to the implantation chamber. The uterine glands were few in number and located away from the implantation chamber.

Cells of the uterine glands contained moderate to large amount of proteins, RNA -containing particles and a small to moderate amount of polysaccharides. In most of the specimens examined, the lumina of the uterine glands did not display any secretory material. These observations led to the assumption that the decidua, and not the uterine glands, play an important role in the nutrition of the developing rat embryo before placentation is completed.

INTRODUCTION

The decidua around the developing embryo in rodents is an organized structure in the centre of which the implantation chamber is found. This structure occurred in response to a stimulus given to the uterus at the time of attachment of the blastocyst to the luminal epithelium (McLaren, 1969, Finn 1971, Hinchliffe and El-Shershaby, 1975). Lobel *et al.* (1965) described two types of decidual cells in the decidua according to their structure and function. Histochemically, glycogen, lipids

* *Present Address :* Faculty of Science, Qatar University, Doha, Qatar.

and some enzymes were demonstrated in the uterine cells of some mammals during pregnancy (Christie, 1966 & 1967, Wong and Dickson, 1969). However, little information is known concerning the distribution and concentration of proteins, RNA and polysaccharides in the constituent cells of the uterus of pregnant rodents.

The aim of the present investigation was to study the development of the decidua around the early embryo of the rat '*Rattus rattus*' and to elucidate the nutritive role of the decidua and the uterine glands during and after implantation of the rat embryo.

MATERIALS AND METHODS

Fifty sexually mature females of albino rat '*Rattus rattus*' were used in the present study. Every three females were caged with a mature male and the day of pregnancy was considered when the vaginal plug was found. The implantation sites were identified by the technique of Psychoyos (1961). Implantation sites for this study were obtained at 129, 138 and 153 h p.c. (hour post-coitum) and at days 8 and 9 of pregnancy. The sites of implantation were fixed in Bouin's fluid for 24 hours, and in Carnoy's fluid for three hours. Then specimens were dehydrated in ethanol, cleared in xylol and embedded in paraffin wax. Transverse sections of 5-7 μ thick were prepared and stained with haematoxylin and eosin for general histological observations. The mercury bromophenol blue technique by Mazia *et al.* (Pearse, 1963) and toluidine blue method of Kramer and Windrum (1955) were used for demonstrating general proteins and RNA-containing particles, respectively. The periodic acid schiff (PAS) of Hotchkiss (1948) was applied to demonstrate polysaccharides in the uterine tissue. Diastase treated sections were also used (1% aqueous at 37°C for one hour) to verify the presence of glycogen and to find out whether there is any PAS positive material than glycogen exist in these cells.

RESULTS

1 - The Decidue

i - Histological observations

Histological examination of the pregnant uteri at 129 h p.c. stage showed that the subepithelial stromal cells lying very near to the implantation chamber were changed in form from the elongated fibroblast to the decidual rounded or oval cells (Figs. 1 & 2). These cells were characterized by a moderate eosinophilic cytoplasm

and large strong by basophilic nuclei. The nuclei showed an accumulation of chromatin material at their peripheries. Each nucleus contained one to three, and sometimes five, basophilic nucleoli. Few binucleate decidual cells were observed near the uterine epithelium (Fig. 1). At 138 h p.c. stage, cells of the decidual tissue formed a distinct basophilic cup around the antimesometrial uterine epithelium (Fig. 3). At this stage, an increase in number of the decidual cells and in the nuclear size was observed in comparison with 129h p.c. stage. At 153h p.c. stage the subepithelial stromal cells underwent full decidualization. The enlarged decidual cells were more pronounced at this stage than 138h p.c. stage (Fig. 5). These large decidual cells were packed together and their cytoplasm was strong by basophilic. The nuclei became large and their outlines were irregular. At day eight of pregnancy, the decidual tissue was more developed and appeared similar in form to 153h p.c. stage (Fig. 5).

At day nine of pregnancy, hypertrophy and hyperplasia of the decidual cells were more pronounced (Fig. 6). The embryo at this stage was more developed and the main embryonic layers were differentiated. The large decidual cells became in intimate contact with each other and in close association with the developing embryo. At this stage, the cytoplasm was moderately stained but the nuclei were densely stained. The binucleate cells increased progressively in number from 129h p.c. to day nine of pregnancy. These cells were similar in structure to the adjacent decidual cells (Figs. 4 & 6).

ii - Histochemical observations

a - Bromophenol Blue Preparations

At 129h and 138h p.c. stage the decidual cells exhibited a strong blue colouration (Fig. 7). The proteinic material was in the form of very small granules scattered in the cytoplasm and in the intracellular spaces. The nuclei were also deeply stained. A marked increase in the reactive materials was observed in the decidual tissue from 129h p.c. to 138h p.c. stage. The fully decidualized cells at 153h p.c. stage were deeply stained indicating their high content of proteins (Fig. 3). A homogeneous deep blue colouration was observed inbetween the decidual cells very near to the antimesometrial region of the implantation chamber. A similar observation was recorded in the decidua at day eight of pregnancy. The nuclei contained small dispersed proteinic granules. The nucleoli were strongly stained with bromophenol blue (Fig. 9).

b – Toluidine Blue Preparations

The decidual cells at stages 129h p.c. and 138h p.c. were found to contain a considerable amount of RNA-containing particles in their cytoplasm and in the nucleoli (Fig. 10). A slight increase in the RNA contents was observed in the decidua at 153h P.C. stage. Also, a very dense toluidine blue colouration was shown near the implantation chamber (Fig. 11). Similar results were found in the decidual tissue at day 3 of pregnancy. At this stage the nucleoli showed more dense blue colouration than the cytoplasm (Fig. 12).

c – PAS Preparations

Cells of the decidua at 129h p.c. stage showed moderate to strong PAS reaction (Fig. 13). However, an increase in the polysaccharide contents of the cells was observed at 138h p.c. stage (Fig. 14). A strong PAS reaction was found in the intercellular spaces antimesometrially to the implantation chamber. At 153h p.c. stage and on the day 8 of pregnancy, small granules, positively stained for polysaccharides, were observed in the cytoplasm of the decidual cells (Fig. 15 & 16). The decidua showed deep pink colouration, near the developing embryo, indicating its high content of polysaccharides. After diastase treatment most of the PAS – positive material was removed, and only faintly stained meshwork was observed.

2 – The Uterine Glands

i – Histological observations

The uterine glands in all stages examined (129h, 138h and 153h p.c.) were few in number and located nearer to the muscular layer (Figs. 17, 18 & 19). The wall of the uterine gland consisted of cuboidal or low columnar epithelial cells. The cytoplasm of these cells was less eosinophilic than that of uterine epithelial cells. The basophilic nuclei were basically or centrally located in the cytoplasm and contained one or two dense nucleoli. The chromatin granules were distributed all over the nuclei and / or near the nuclear membranes. At 133h p.c. stage there was an increase in number of the uterine glands but they were still displaced away from the uterine lumen (Fig. 18). Cells of the uterine glands, at 138h p.c. stage, showed very small basophilic granules in their apical regions. No appreciable amounts of glandular secretion was noticed in the gland lumina. However at 153h p.c. a very small amount of glandular secretion could be observed in the lumina of some glands (Fig. 19).

ii - Histochemical observations

a - Bromophenol Blue Preparations

Cells of the uterine glands at 129h p.c. and 138h p.c. stages were strongly to deeply stained indicating their high contents of general proteins. The luminal apices of these cells exhibited a deep stainability (Fig. 20). The nuclei of the gland cells appeared strongly stained containing tiny blue granules and densely stained nucleoli. At these two stages, no proteinic secretion was found in the lumina of the glands. Later, at 153h p.c. stage, the gland cells were moderately to strongly stained and their nuclei contained strongly coloured proteinic granules (Fig. 21). The lumina of some glands at 153h p.c. contained a small amount of proteinic secretions (Fig. 21).

b - Toluidine Blue Preparations

A deep toluidine blue reaction was observed in the gland cells, at 129h p.c. and 138h p.c. stages, indicating their high contents of RNA (Fig. 22). However, at 153h p.c. stage, the gland cells were moderately stained (Fig. 23).

c - PAS Preparations

At 129h p.c. stages cells of the uterine glands displayed a moderate PAS reaction (Fig. 24). The luminal surfaces of these cells were moderately to strongly stained. Also, moderate to strong PAS pink colouration was observed in the uterine gland cells at 153h p.c. stage (Fig. 25). However, some gland cells were negatively stained. The lumina of the uterine glands showed no PAS stained material. Sections pre-treated with diastase showed faintly stained polysaccharide materials.

DISCUSSION

In the present study, the development of the decidua around the implantation chamber in the rat was followed. The stromal cells were transformed into round basophilic decidual cells. The decidual tissue was observed at 129h p.c. stage and growth of the decidua appeared to take place accompanying development of the embryo and reached a maximum at day nine of pregnancy. The decidual cells showed basophilic cytoplasm and large nuclei. At 138h P.C. stage the decidua form a distinct basophilic area around the antimesometrial uterine epithelium. At this stage, an increase in number and size of the decidual cells was observed and hypertrophy and hyperplasia were observed in this tissue at day nine of pregnancy.

The presence of large nuclei and prominent nucleoli (one to five densely basophilic nucleoli) in the decidual cells could be considered as a sign of increased synthetic activity in the decidua. The fully transformed and differentiated decidual cells were packed and attached together to form a solid mass of cells around, and in direct contact with the developing embryo. Similar observations were reported in the uterus of pregnant mice by Finn and Lawn (1967). The adhesion of the decidual cells described in the present study may be regarded as a means to allow the passage of materials between them. This observation is in agreement with the suggestion of Loewenstein and Penn (1967) that normal tissue growth and differentiation depend on the flow of materials from the interior of one cell to another through the junctional cell surfaces.

Histochemically, cells of the decidua in all stages examined were noticed to contain a large amount of proteins, RNA-containing particles and polysaccharides. A dense homogeneous secretory material of proteinic-polysaccharide nature was observed inbetween the decidual cells and very near to the implantation chamber. The concentration of RNA in the examined decidual cells was more or less similar to their contents of general proteins. This situation is usually found in cells concerned with protein synthesis. Inoue (1971) described the presence of proteinic material in the stromal cells during the preimplantation period in the rat and El-Banhawy *et al.* (1982) showed the presence of a large amount of proteins in the decidual cells of the mouse uterus during early pregnancy. Also, an increase in the RNA contents was reported in the enlarged decidual cells in the rat (Lobel *et al.*, 1965) and in the rabbit (Christie, 1966).

The increase in the amount of polysaccharides observed in the decidua, in the present study, between 129h p.c. stage to 153h p.c. stage was similar to that found in the mouse uterus during early pregnancy (Finn and Hinchliffe, 1965). Glycogen was reported in the decidua around the implantation chamber in the rat and golden hamster (Christie, 1966, Parkening and Soderwall, 1974). Foster *et al.* (1963) working on the pregnant hamster, postulated that one function of the glycogen in the implantation region might be providing a source of energy for the developing embryo. Christie (1966) described the distribution of non-specific and specific phosphatases in the developing decidua of the rabbit and suggested that the decidua acts as a supplier of energy to the embryo. Danielli (1953) suggested that alkaline phosphatase was associated with secretion and with the formation of fibrous proteins. Finn (1971) and El-Shershaby (1974) revealed that the decidual cells displayed clear signs of activity demonstrated by their high contents of endoplasmic

reticulum. This finding was in agreement with the increase in the decidual secretion observed in the present study.

The uterine glands examined in the present work were few in number and located away from the implantation chamber. The gland cells showed high proteinic and RNA contents and small to moderate amounts of polysaccharides. On the other hand, the gland lumina, in most specimens studied, showed no glandular secretion. Similar results were obtained from the mouse by El-Banhawy *et al.* (1982). A limited amount of secretions, faintly stained for proteins and polysaccharides, was observed in the lumina of few glands. Thus, it was assumed that the uterine glands in the studied embryonic stages played a slight or no role in nourishing the developing rat embryo.

The decidual secretion observed in the present material might be used as a nutrient by the developing rat embryo before formation of the placenta. Kaldas (1983) showed that the uterine epithelium in the pregnant rat, at 153h p.c. completely shed from around the rat egg - cylinder and the trophoblast cells were found in close association with the decidual cells. Similar observations were described in the mouse by El-Shershaby and Hinchliffe (1975) and in the golden hamster by Parkening (1976). In the present study, a considerable amount of decidual secretion was observed at 153h p.c. and days eight and nine of pregnancy, near the antemesometrial trophoblast cells. The decidual secretion could enter the trophoblast cells with great facilities by diffusion as only a narrow space was found to separate the maternal from the embryonic cells. From the findings of the present study and from the above interpretations, it was concluded that the decidua played an important role in nourishing the developing rat embryo. This conclusion is in agreement with Amoroso's study (1952) who reported that in most species with poor decidualization, the uterine glands supply copious uterine milk to the embryo, whereas in species with much decidualization, the glands are usually non-functional after implantation.

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LEGEND OF PLATES AND FIGURES

Plate I

Figures 1-4 : Haematoxylin and eosin stained sections for histology, showing intact uterine epithelium (ep) and round decidual cell. Few binucleate cells are observed in the stroma (s) at arrows. The embryo (tr) is found in the uterine lumen (ul). Figs. 1 & 2 at 129h p.c. (x 600 & 950), and Figs. 3 & 4 at 138h p.c. (x 900 & 1000).

Figures 5 & 6 : Haematoxylin and eosin stained sections for histology at 153h p.c. and at day nine of pregnancy, showing, large round decidual cells. Binucleate decidual cells are observed at arrows. (X 1100 & 1000).

Plate II

Figures 7-9 : Bromphenol blue preparations for general proteins in the uterine epithelium (ep) and in the decidua (dl). Arrows represent decidual secretion. Fig. 7 at 138h p.c. (X 3000), Fig. 8 at 153h p.c. (X 1500). and Fig. 9 at day eight of pregnancy (X 1600).

Histological and histochemical studies

- Figures 10 – 12* : Toluidine blue preparations for ribonucleic acid (RNA) in the uterine epithelium (ep) and in the decidua (dl) at 129h p.c., 153h p.c. and day eight of pregnancy, respectively. (X 1100, 1500 & 1200).
- Figures 13 – 16* : PAS preparations for polysaccharides in the uterine epithelium (ep), in the embryo (icm & tr) and in the decidua (dl) at 129h p.c. and day eight of pregnancy, respectively, (X 1500).

Plate III

- Figures 17 – 19* : Haematoxylin and eosin stained sections for histology, showing uterine glands at 129h p.c., 138h p.c., and 158h p.c. respectively. (X 700, 800 & 1000).
- Figures 20 & 21* : Bromphenol blue preparations for general proteins in the uterine glands at 138h p.c. stages. (X 1500).
- Figures 22 & 23* : Toluidine blue preparations for ribonucleic acid (RNA) in the uterine glands at 129h p.c. and 153h p.c. stages. (X 1500).
- Figures 24 & 25* : PAS preparations for polysaccharides in the uterine glands at 138h p.c. and 153h p.c. stages. (X 1500).

Plate - I

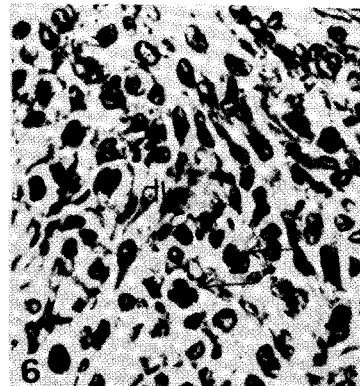
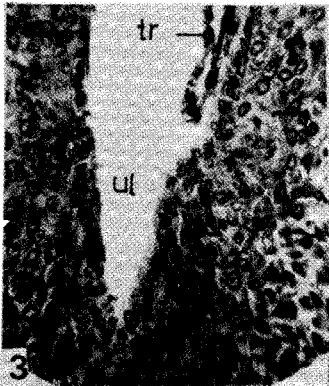
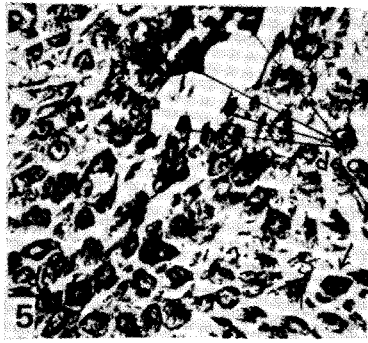
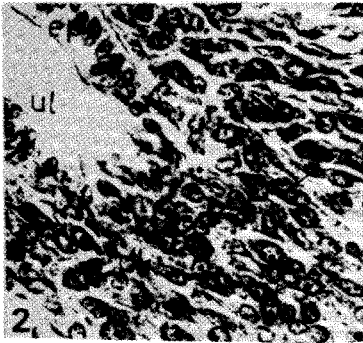
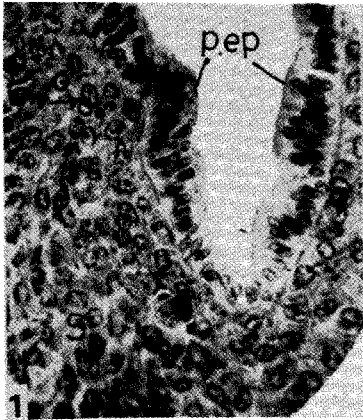


Plate – II

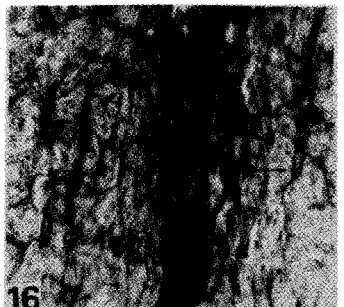
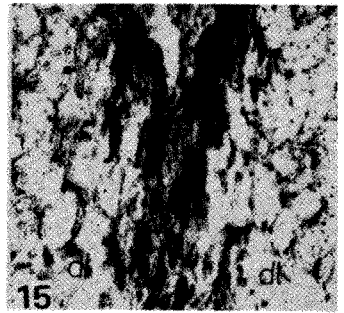
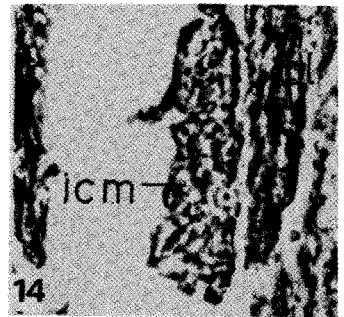
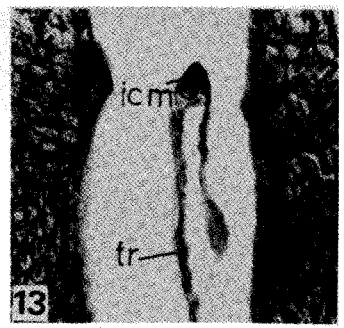
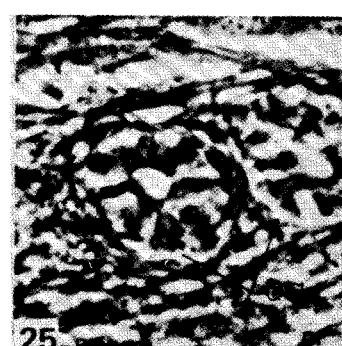
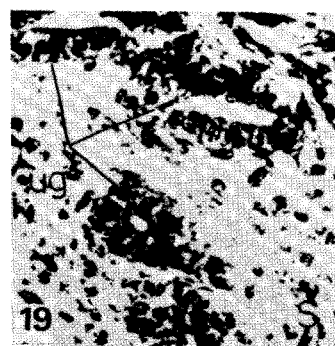
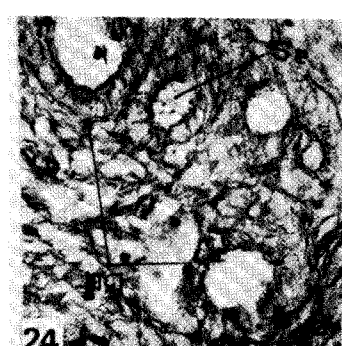
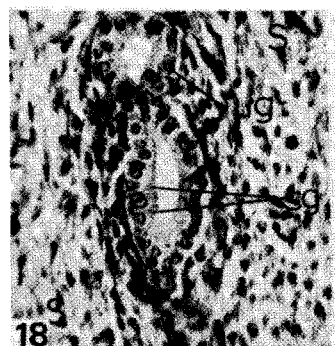
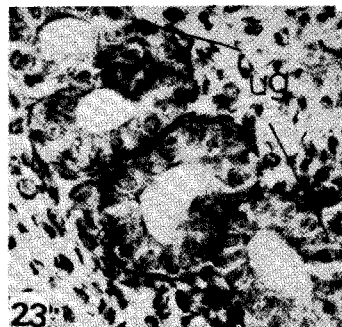
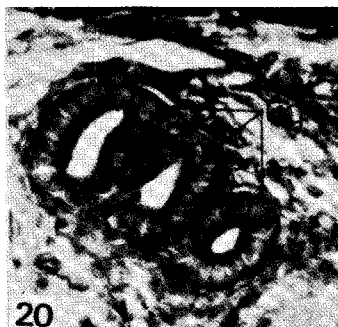
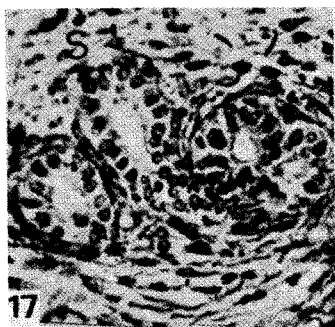


Plate - III



ABBREVIATIONS

pc = post - coitum
 bm = basement membrane
 dec = dead epithelial cells
 dl = decidua
 ep = epithelium
 icm = inner cell mass
 le = loose epithelium

pep = pseudostratified epithelium
 s = stroma
 se = secretion
 sg = secretory granules
 tr = trophoblast cells
 ug = uterine glands
 ul = uterine lumen.

دراسات هستولوجية وهستوكيميائية على نسيج الحشوة وغدد الرحم أثناء الحمل في الجرذ

عبد الفتاح محمود الشرشابي و أنور الحسيني العجمي

و

سامية قلندس

يتناول هذا البحث دراسة هستولوجية وهستوكيميائية لنسيج الحشوة وغدد الرحم حول الجنين النامي في خمس مراحل جنينية عند ١٢٩ ، ١٣٨ ، ١٥٣ ساعة عقب الإخصاب وكذلك اليوم الثامن واليوم التاسع من الحمل في الجرذ ، وقد توصل البحث إلى النتائج التالية :

يتكون نسيج الحشوة حول الجنين النامي - عند ١٢٩ ساعة عقب الإخصاب - من تحول خلايا الاستروما الليفية الشكل إلى خلايا دائرية ذات أنوية كبيرة ، ويبلغ هذا التحول مداه في اليوم التاسع من الحمل حيث يلاحظ زيادة كبيرة في عدد وحجم خلايا نسيج الحشوة ، وتتميز هذه الخلايا بالصبغة القاعدية ووجود أكثر من نوية (١ - ٥) في النواة الواحدة .

لوحظت زيادة كبيرة في محتوى البروتينات والحامض النووي (RNA) وعديد السكريات بخلايا نسيج الحشوة من مرحلة ١٢٩ ساعة إلى ١٥٢ ساعة عقب الإخصاب ، وقد أوضحت الدراسة أيضاً وجود افراز كثيف غني بالبروتينات وعديدات التسكر بنسيج الحشوة وقريب من طلائية الرحم عند المراحل الجنينية ١٢٩ ساعة ، ١٣٨ ساعة عقب الإخصاب ، وبعد تلاشي الطبقة الطلائية عند مرحلة ١٥٣ ساعة من الإخصاب توجد المادة الافرازية ملاصقة للخلايا الجنينية الاغتذائية .

أوضحت الدراسة بُعد غدد الرحم عن مكان التصاق الجنين برحم الأم ، وأن خلايا هذه الغدد تحتوي على كمية كبيرة من البروتينات والحامض النووي (RNA) وعلى كمية قليلة من عديد السكريات ، ومن ناحية أخرى أوضحت الدراسة الهستوكيميائية عدم وجود افرازات داخل تجاويف غدد الرحم .

وبمناقشة نتائج هذا البحث مع بحوث مشابهة على حيوانات أخرى فإنه يوضح أن نسيج الحشوة - في حيوانات التجارب المستعملة بالبحث - يلعب دوراً رئيسياً في تغذية الجنين النامي قبل تكوين المشيمة .