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# SOME HISTOLOGICAL AND HISTOCHEMICAL ASPECTS OF EGG-IMPLANTATION IN THE RAT

By

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## ABSTRACT

The rat embrayo and uterine epithelium were studied during implantation at 129h p.c., 138h p.c. and 153h p.c., stages. The basophilic bodies found in the inner cell mass and in the uterine epithelium represent dead cells with a dense stained cytoplasm and pyknotic nuclei. However, these bodies found in the trophoblast cells represent phagocytosed dead or degenerated epithelial cells that expelled from the uterine epithelium toward the blastocyst. All these bodies are strongly stained for protienss, DNA, and polysaccharides.

A variable content of protiens, RNA, and DNA was reported in the trophoblast and inner cellular mass as well as in all layers of the embrayo during the implantation stages. However, an increase in polysaccharides was observed during lare stage (153h p.c.) in ther rat egg-cylinder, suggesting that the embrayo consumes the polyccharides as an energy sourse material during the period of blastocyst implantation as well as during transformation into the egg-cylinder.

Cells of the uterine epithelium showed certain features of degeneration such as vacoulation, shrinkage and pyknotic nuclei. Also, a decrease in the protien, RNA, and polyccharide contents of the uterine epithelium was observed. These changes take place during the whole break down of the uterine epithelium which occured at the late period of implantation (153h p.c., stage).

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## INTRODUCTION

The morphology of implantation of the mammalian embryos within the uterine epithelium has attracted the attention of many authors: in mouse (Finn and McLaren, 1967; El–Shershaby and Hinchliffe, 1974), in rate (Enders and Schlafke, 1967; Zybina, 1976), goldenhamster (Parkening, 1976), in rabbit (Hesssel–dahl, 1971) in bat (Bhiwgade, 1976) and in rhesus monkey (Enders and Schlafke, 1981). These authors showed that implantation is accompanied by degradation of the uterine epithelium. However, some aspects of implantation in the rat related to cell death and phagocytosis need more investigations. Historically, however, less information is available about the metabolic changes that taking place in the embryo and the uterine epithelium during implantation (Finn and Hinchliffe, 1965; Parkening, 1976; El–Banhawy *et al.*, 1982; and El–Shershaby *et al.*, 1986).

In a view of interest in the mechanism of implantation in the rat, it seems valuable to perform an investigation dealing with cell death and phagocytosis. Also, with some events of metabolic changes of protiens, ribonucleic acid (RNA), deoxyribonuleic acid (DNA) and polysaccharidese in the rat embrayo and the surrounding epithelium over the period of implantation and immediately after implantation.

## MATERIALS AND METHODS

Sixty pregnant females of albino rat *Rattus rattus* were used in the present study. Pregnancy was identified as described previously by Kaldas (1983). The technique of Psychoyos (1961) was used to identify the sites of implantation. Stages of pregnancy chosen for this study were obtained at 129h, 138h and 153h p.c. (postcoitus). Implantation sites at these stages were fixed in Bouin's fluid for 24 hours and in Carnoy's fluid for three hours. Parraffin wax sections were prepared and stained with Harris haematoxylin and eosin for general histology.

For histochemical identification of some biological materials in the uterine epithelium and in the embryonic cells, the following methods were used: the mercury bromophenol blue technique of Mazia *et al.* (Pearse, 1968) for general protiens, the toluidine blue nethod of Kramer and Windrum (1955) for RNA–containing articles, the Feulgen reaction (Pearse, 1968) for DNA and the periodic acid Schiff (PAS) of Hotchkiss (1948) for polysaccharides.

## RESULTS

### I. Histological Observations

#### 1. The Embryo

Structure of the rat embryo described in the present study was more or less similar to that of the mouse previously described by some authors in a comparable stages of development (Finn and McLaren, 1967; El-Shershaby and Hinchliffe, 1974; El-Shershaby, 1984). At 129h p.c. and 138h p.c. stages, some lateral and antimesometrial trophoblast cells contain faintly stained fine basophilic granules as well as cytoplasmic vacuoles (Figs. 1 & 3). These bodies represent dead or degenerated epithelial cells which were expelled from the uterine epithelium toward the trophoblast cells. In the inner cellular mass, two or three dead or motibund cells with basophilic and pyknotic nuclei were observed (Fig. 4).

At 153h p.c. stage, the blastocyst developed into an elongated egg-cylinder (Fig. 2). In this condition, the inner cellular mass grow to form a protrusion of cells, whose outer surface was covered by a layer of endodermal cells (Fig. 2 & 5). This mass of cells was found to differentiated into two distinct masses of cells: an extra-embryonic ectodermal mass and an embryonic ecrodermal mass. Antimesometrially, the trophectodermal cells of the egg-cylinder extended between the degenerated epithelial cells. Within some of these trophectodermal cells, some dense basophilic and phagocytosed dead cells were identified. Figure (5) shows few disrupted endodermal cells that were displaced away from the inner cellular mass to the yolk sac cavity. Also, in the ectoderm, cells with shrinkage vacoulated cytoplasm and dense basophilic nuclei were identified.

#### 2. The Uterine Epithelium

At 129h p.c. stage, the epithelium of the implantation chamber was consisted of low and tall columnar cells (Fig. 6 & 7). Antimesometrially, the uterine epithelium was arranged in a pseudostratified layer (Fig. 6). At 138h p.c., stage the uterine epithelium of the mesometrial half of the implantation chamber was consisted of cuboidal and elongated epithelial cells (Figs. 1 & 2). At this stage, a further decrease in the cytoplasmic area was observed when compared with

the previous stage. At 153h p.c., stage the whole uterine epithelium disappeared from the embryo. In some specimens, flattened epithelial cells were identified around the mesometrial half of the embryo (Figs. 2 & 8). For more histoloigical details of the embrayo, uterine epithelium and cells, see Kaldas (1983).

## II. Histochemical Observations

In this part the depth of colour produced by the cells was taken as an indicator for the amount of the bioligical materials present in that cell.The term faint, weak, moderate, strong, and deep used in the present study refered to the relative amounts of the materials present. Thus, faint refered to the least amount and deep to the greatest amount.

### 1. The Embryo

#### i. General Protiens

At 12h p.c., stage , the trophoblast cells contain moderate to large amount of proteins (Fig. 7). The cytoplasm of these cells showed a homogeneous proteinic material. The nuclei were moderately stained but the nucleoli were strongly stained with bromophenol blue. The phagocytosed degenerated epithelial cells described in the histology part (Fig. 7), exhibited strong to deep blue colouration. At 153h p.c., stage, the trophectodermal cells of the egg–cylinder showed moderate to high content of proteins (Fig. 8). The proteinic content of these cells was represented by a diffuse blue colouration and small granular particles positively stained with bromophenol blue.

Cells of the inner cellular mass, at 129h and 138h p.c. stage, showed moderate to strong stained proteinic materials in their cytoplasm and nuclei (Fig. 7). The embryonic mass of the egg–cylinder at 153h p.c. stage showed more proteinic materials than 129h and 138h p.c., stage. Cells of the endoderm, extra–embryonic ectoderm and embryonic ectoderm exhibited a moderate to strong bluish reaction (Fig. 8). A strong stained strip of proteinic nature was observed at the nuclear periphery. Also, proteinic granules were found in the chromatin material within the nuclei (Fig. 8).

#### ii. Ribonuclei Acid (RNA)

A positive toluidine blue straining particles were observed at 129h p.c. and 138h p.c. stage in the cytoplasm and nucleoli of the trophoblast cells and in the

inner cellular mass cells (Fig. 9). The cytoplasm of the inner cellular mass cells showed greater amount of RNA than that of the trophoblast cells, a picture which is similar to the proteinic content of both types of cells (Fig. 9). At 153h p.c., stage the embryonic cells of the rat egg-cylinder were found to contain moderate to high content of RNA containing partiucles (Fig. 10).

**iii. Deoxyribonucleic Acid (DNA)**

At 129h p.c., and 138h p.c., stages the nuclei of the trophoblast cells as well as of the inner cellular mass were moderately stained by Feulgen reaction. The DNA content was represented by small elements homogeneously distributed in the nuclei (Fig. 11). The nuclei of the dead inner cellular mass were deeply stained by Feulgen reaction. In the rat egg-cylinder, a moderate amount of DNA was also observed in the nuclei of the trophectodermal cells. Cells of the endoderm, extra-embryonic ectoderm and embryonic ectoderm showed a moderate to strong Feulgen reaction in their nuclei indicating their moderate to high content of DNA.

**iv. Polysaccharides**

The trophoblast cells at 129h p.c. and 138h p.c., stages showed small to moderate amount of PAS positive material. The polysaccharide content of these cells was represented by fine granules. Also, a diffuse pink colouration was observed in the cytoplasm (Fig. 12). The phagocytosed bodies found in the trophoblast cells were deeply stained with PAS reaction (Fig. 14).At 153h p.c. stage, cells of the trophectoderm were found to contain moderated amount of polysaccharides. On the other hand, a slight increase in the polysaccharide content of the inner cellular mass cells was observed at 138h p.c., than 129h p.c. stage (Figs. 12 & 13). The embryonic layers of the egg-cylinder at 153h p.c., contain moderate to a rather high content of polysaccharides (Fig. 15 & 16). Cells of the endoderm showed PAS positive staining granules together with a diffused stained material. Also, a deep positive PAS reaction was observed at the cell surfaces and at the endodermal basement membrane. The embryonic and extra-embryonic ectodermal cells showed strong pink colouration indicating their high content of polysaccharides. In this condition, the polysaccharides were represented by a diffussed material and fine of coarse granules positively stained with PAS reaction.

**2. The Uterine Epithelium**

**i. General Proteins**

A moderate to high content of prteins was observed , at 129h p.c. and 138h p.c., stages, in the uterine epithelium around the rat blastosyst. The luminal apices of the epithelial cells were strongly to deep stained with bromophenol blue. However, the basal region of some of these cells were moderately stained. Few epithelial cells at the antimesometrial region of the implantation chamber were vacuolated and exhibited weak of faint blue colouration (Fig. 17). Generally, The proteinic material of the epithelial cells was gradually decreased from 129h p.c. to 138h p.c. and to 153h p.c. stages.

**ii. Ribonuclei Acid (RNA)**

At 129h p.c. and 138h p.c. stages, a weak to moderate toluidine blue reaction was observed in the cytoplasm of the epithelial cells. The nucleoli of these cells showed faint or negative reaction (Fig. 18) indicating that the protein synthesis in such cells was low or completely inhibited. This last observation indicates a marked change in the RNA containing particles takes place during the break down of the uterine epithelium around the developing embryo.

**iii. Deoxyribonucleic Acid (DNA)**

Concerning the DNA content of the uterine epithelium around the blastocyst at 129h p.c. and 138h p.c., stages, it has been found that the nuclei of the epithelial cells contain moderate to high content of DNA (Fig. 19). However, the dead epithelial cells showed deeply nuclei where the DNA containing particles were diffused in the nucleoplasm.

**iv. Polysaccharides**

At 129h p.c., stage small to moderate amount of polysaccharides was observed in the epithelial cells as indicated by positive PAS. Some vacuolated epithelial cells with faint or weak PAS were also identified (Fig. 12). At 138h p.c. stage, the uterine epithelium showed very faint positive PAS reaction indicating its low content of polysaccharides. On the other hand, the basement membrane was deeply stained. The degenerated epithelial cells, found at the anti-mesometrial region of the egg–cylinder at 153h p.c., stage showed faint to weak positive PAS reaction (Fig. 20).

## DISCUSSION

The present investigation was suggested to study some aspects of implantation of the rat embryo in the uterine wall at 129h, 138h and 153h p.c., stage by using some histological and histochemical procedures. The nature of

the basophilic bodies was observed in the uterine epithelium, in the trophoblast cells and in the cells of the inner cellular mass.

The present study shows that implantation of the rat blastocyst in the uterine wall is similar to that observed in other rodents (Finn and McLaren, 1967; Parkening, 1976). Some epithelial cells around the blastocyst at 138h p.c. stage were degenerated clearly and phagocytosed by the facing trophoblast cells. By 153h p.c. stage, the uterine epithelium disappeared completely or partially from the developing embryo. The egg-cylinder become directly in contact with and gradually embedded in the subepithelial stromal tissue.

In the present study, dense basophilic bodies were observed at 138h p.c., stage in the uterine epithelium, in the trophoblast cells and in the inner cellular mass. The dense bodies of the uterine epithelium and the inner cellular mass represent the isolated dead cells with densed and shrinked cytoplasm and pyknotic nuclei. These dead cells showed strong reaction for proteins, DNA and polysacchrides. However, the dense bodies of the trophoblast cells were deeply basophilic. The latter bodies were similar to that bodies described in the mouse by Finn and Lawn, 1968; and El-Shershaby and Hinchliffe, 1974. The dead cells were probably expelled to the uterine lumen where they have been phagocytosed by the trophoblast cells.

The basophilic dead cells found in the inner cellular mass were most probably engulfed by the adjacent normal cells, a similar observation was previously described in the mouse by El-Shershaby, 1981. These dead inner cells did not described by Tachi *et al.*, (1970) in a similar stage of implantation in the rat blastocyst. These authors described also, some inclusions and dense signet-ring shaped bodies in the inner cellular mass at day 5 p.c., 24h stage. The authors explained these structures as unidentified inclusions and materials in the inner cellular mass. On comparing the structures of Tachi *et al.*, (1970) with the basophilic bodies found in the mouse (Finn and Lawn, 1968; and El-Shershaby and Hinchliffe, 1974), it was shown that they represent digested inclusions and dead cells in the inner cellular mass of the rat blastocyst. Similar basophilic dead cells were observed in the blastocyst of the golden hamster (Parkening, 1976) and during in vitro differentiation of the mouse blastocyst in tissue culture (Wilson and Jenkinson, 1974). The dead cells of the inner cellular mass described in the present study may represent spontaneous degenerated cells during differentiation of the inner cellular mass, as well as cells of chromosomal abnormalities might be the source of these dead cells (Glucksmann, 1965; Forsberg and Kallen, 1968). Although, phagocytic vacuoles

did not successfully identified in the present study, yet on the bases of cell death and phagocytosis described in some necrotic tissues (Ballard and Holt, 1968; Dawd and Hinchliffe, 1971), these results might be interpreted as a process of ingestion and digestion of dead cells by their normal neighbouring cells of the inner cellular mass.

In 138h p.c., stage, the uterine epithelium with a shrinkage and less eosinophilic cystoplasm were observed. The polysaccharide and protein contents of these cells were clearly decreased between 129h and 138h p.c., stages. Also, these epithelial cells showed low RNA content. On the other hand, the nuclei of the moribund and degenerated epithelial cells were strongly stained for DNA, a finding which was similar to that reported in the dead cells of the necrotic zone in the rat foot (Ballard and Holt, 1968). All these features may be considered as self degenerative changes in the epithelial cells was presumably an expression of low metabolic cell death. It was shown by a number of authors that autolysis was the process responsible for death of the epithelial cells in necrotic tissues and the lysosomes play a good role in such type of cell death (Enders and Schlafke, 1969; Smith and Wilson,1971). The present results indicate that the degenerated epithelial cells stimulate the trophoblast cells at 138h p.c., stage to phagocytose certain epithelial cells found in the implantation chamber.

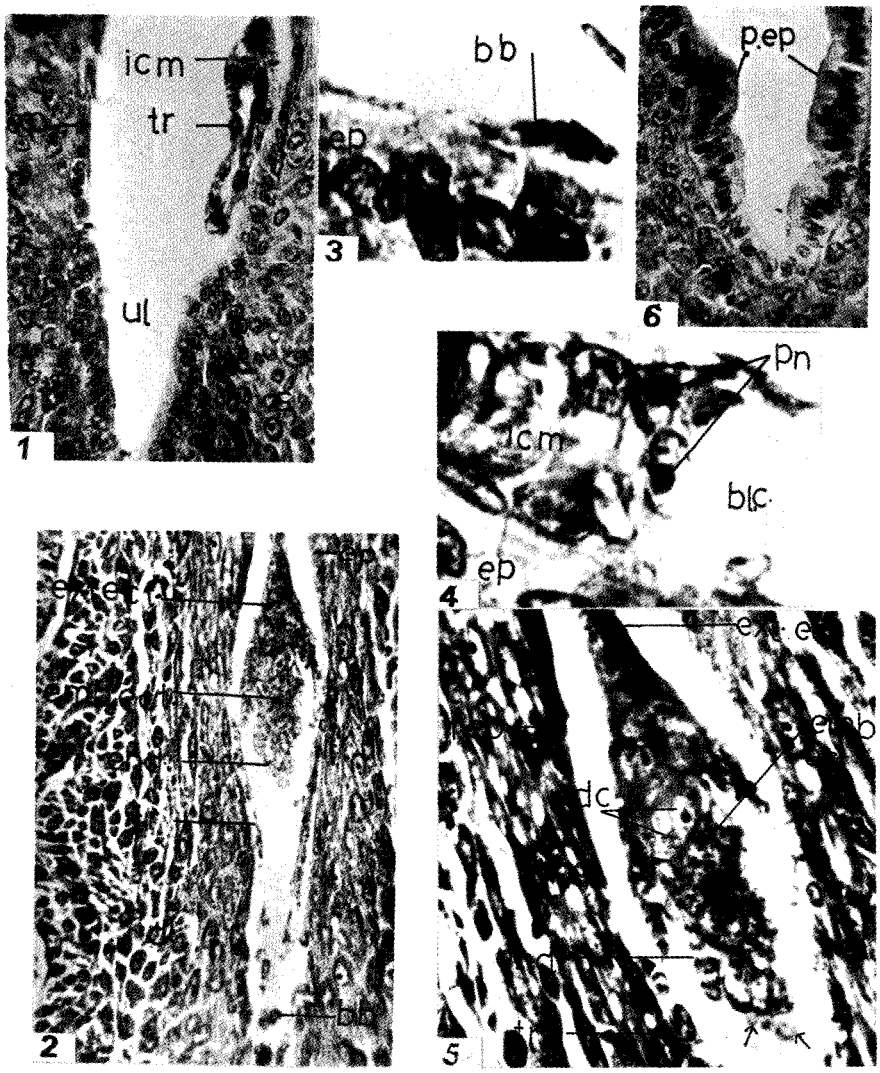
Histochemically, a moderate to high content of proteins, RNA containing particles and DNA was reported in the present study in the trophoblast cells and in the cells of the inner cellular mass between 129h and 139h p.c., stages. Also, an increase in the amount of these biological materials was observed in the embryonic layers of the implanting embryo at 153h p.c. stage. The presentd results suggest that the increase in the protein synthesis from the blasocyst stage to the egg–cylinder stage possibly coinciding with the first major use of RNA templates from the embryo own genome. similar findings were reported in the mouse blastocyst by McLaren (1972), Weitlauf (1973) and El–Banhawy *et al.*, (1982). The latter authors (El–Banhawy *et al.*, 1982) recorded an increase in the protein content of the mouse blastocyst accompanied by development for 105h p.c. to 119h p.c. stages. On the other hand, Zybina (1964) described ribonucleic acid in the cleaving ova and in all stages of early embryonic development in rabbits. Zavarzin *et al.*, (1966) studied the incorporation of $H^3$–thymidine in early embryogenesis of the mouse between 4th to 10th day of pregnancy. The authors observed an increase in $H^3$–thymidine incorporation during development of the mouse embryo from 4 to 5 and 10 indicating an increase in DNA synthesis.

The present results indicate a low content of polysaccharides in the rat embryo between 129h p.c. and 138h p.c. stages. During the latter period the implantation of the blastocyst in the uterine wall and the developing it into the egg-cylinder was occured. At 153h p.c., stage the egg-cylinder showed an increase in the polysaccharides content of all embryonic layers. The endodermal cells exhibited a high content of PAS positive material. Similar results were reported in other animals by several authors. In the golden hamster, Parkening and Soderwall (1974) observed a strong PAS reaction in the one-cell stage. El-Banhawy *et al.*, (1982) described a low content of polysaccharides in the trophoblast and inner cellular mass of the implanted mouse blastocyst between 105h p.c. and 113h p.c., stages. Also, a decrease in the glycogen content in rat and mouse blastocyst at various stages of deveopment was reported by Christie (1966) and Thompson and Brinster (1966). The present data of polysaccharides content in the rat embryo support and confirm these of El-Banhawy *et al.*, (1982) on mouse blastocyst. Finally, we can conclude that the rat consume the polysaccharides as an energy source material during kits expansion and transformation into the egg-cylinder form.

## Abbreviations

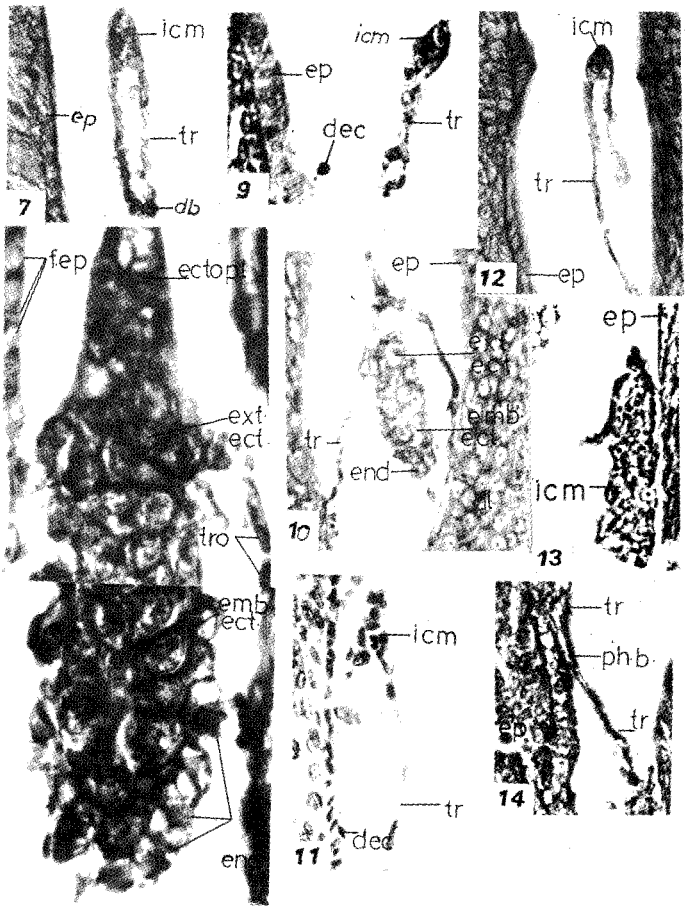
| | | | | | |
|---|---|---|---|---|---|
| bb | = | basophilic body | ecto.pl. | = | ectoplacental cone |
| bl.c. | = | blastocyst cavity | ext.ect. | = | extraembryonic ectoderm |
| bm | = | basement membrane | f.ep. | = | flattened epithelium |
| dc | = | dead cells | icm | = | inner cell mass |
| dec | = | dead epithelial cells | p.ep. | = | pseudostratified epithelium |
| dl | = | decidual cells | ph.b. | = | phagocytosed bodies |
| em.ect. | = | embryonic ectoderm | pn | = | pyknotic nucleus |
| em | = | elongated nucleus | rn | = | rounded nucleus |
| end | = | endoderm | tr | = | trophoblast cells |
| ep | = | epithelium | ul | = | uterine lumen |

PLATE I




Figures 1 – 6: Haematosyline and eosin stained sections for histology, showing blastocsyst, uterine epithelium and rat egg–cylinder. Degenerated endodermal cells are observed at arrows. Figs. 1, 3 & 4 at 138h p.c., (X 800, 3500 & 4000), Figs. 2 & 5 at 153h p.c. (X 900 & 1300) and Fig. 6 at 129h p.c. (X 600).

## PLATE II



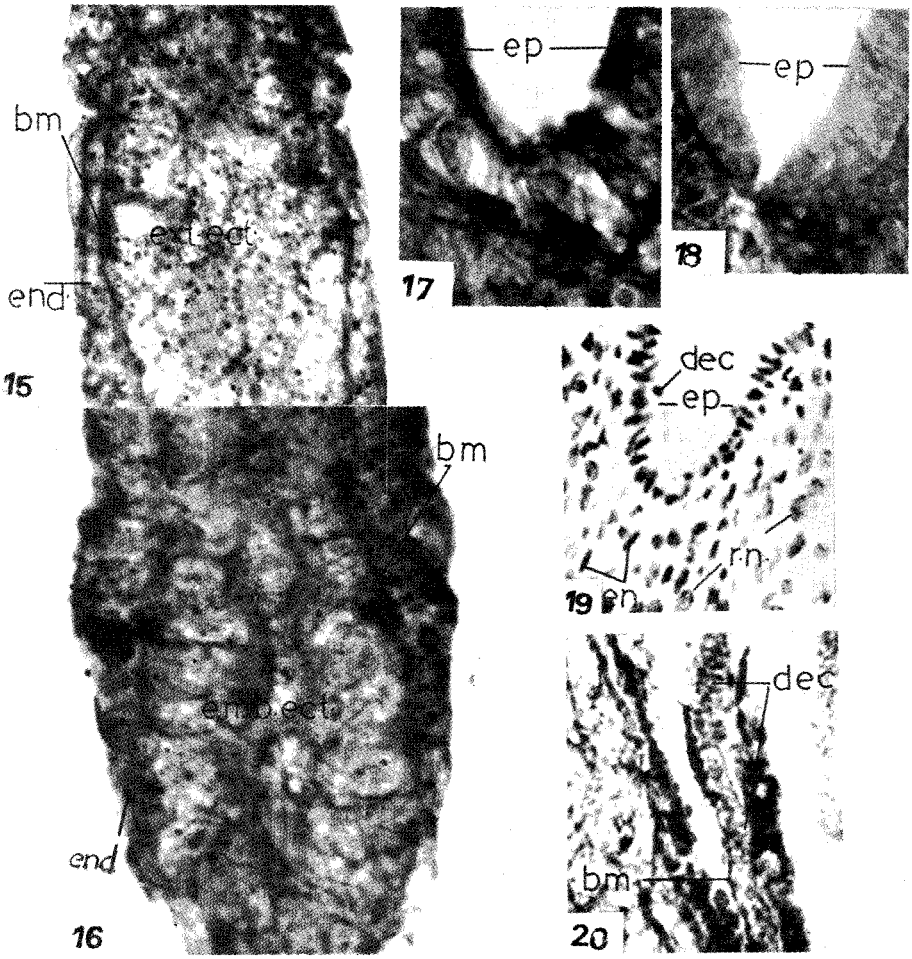

**Figures 7 & 8:** Bromphenol blue reaction for general proteinic materials in the blastocyst at 138h p.c. and egg-cylinder at 153h p.c. (1400 & 3300).

**Figures 9 & 10:** Toluidine blue for RNA-containing particles in blastocyst and egg-cylinder at 138h and 153h p.c. (X 1600 & 1500).

**Figures 11:** Feulgen reaction for DNA in the rat blastocyst at 138h p.c., (x 1700).

**Figures 12, 13 & 14:** PAS positive reaction for polysaccharides in the blastocyst. Fig. 12 at 129h p.c. (X 1500) and Figs. 13 & 14 at 138h p.c. (X 1600 & 1300).

**PLATE III**




**Figures 15 & 16:** PAS positive reaction for polysaccharides in the rat egg–cylinder at 153h p.c. (X 3300).

**Figures 17:** Bromphenol blue reaction in the uterine edpithelium at 138h p.c. (X 3300).

**Figures 18:** Toluidine blue fpr RNA containing particles in the uterine epithelium at 129h p.c. (X 1200).

**Figures 19:** Feulgen reaction for DNA in the uterine epithelium at 139h p.c. (X 1700).

**Figures 20:** PAS reaction in the degenerated epithelium at 153h p.c. (X 2000).

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# بعض المظاهر الهستولوجية والهستوكيميائية لزرع البويضة في الجـرذ

عبد الفتاح محمود الشرشابي - أنور الحسيني العجمي
وسامية كامل قلدس

توصل هذا البحث إلى النتائج التالية :

١ - وجود خلايا متفسخة أو ميتة في الطبقة الطلائية لرحم الأم .
كما تظهر خلايا هذه الطبقة نقصاً شديداً في المحتوى البروتيني لها وفي المواد عديدة التسكـر والأحماض النووية . ويزداد هذا النقص مع تقدم المراحل الجنينية من ١٢٩ ساعة إلى ١٥٣ ساعة بعد الإخصاب إلى أن تتلاشى الطبقة الطلائية تماماً من حول الجنين النامي عند ١٣٥ ساعة بعد الإخصاب .

٢ - ظهور خلايا متفسخة أو ميتة بكتلة الخلايا الجنينية وتمثل هذه الخلايا الميتة بعض الخـلايا الجنينية التي لم تتمكن من التميز إلى خلايا إكتـودرمية أو اندودرمية ، ربما لإحتوائها على شواذ كروموسومات مما يجعلها عرضة للتحـلل والموت .

٣ - يظهر في الجنين النامي زيادة تدريجية في المحتوى البروتيني من ١٢٩ ساعة إلى ١٥٣ ساعـة بعد الإخصاب . ومن ناحية أخرى فإن المواد عديدة التسكر بالجنـين تظهـر نقصـا واضحاً من مرحلة ١٢٩ ساعة إلى ١٣٨ ساعة بعد الإخصاب ، ثم تعود هذه المواد إلى الزيادة الكبيرة عند مرحلة ١٥٣ ساعة بعد الإخصاب .

وبمناقشة هذه النتائج من ناحية النشاط الأيضي للمواد البيولوجية المدروسة بالبحث ، فإنه يستنتج أن هناك عملية تخليق للمواد البروتينية مع نمو الجنين . وفي نفس الـوقت توجد عملية هدم للمواد عديدة التسكر بين ١٢٩ ساعة إلى ١٣٨ ساعة وذلك لإنتاج الطاقة اللازمة للجنين أثناء زرعه بجدار رحم الأم ونموه إلى الجنين الأسطواني .