The Scientific Journal of University of Benghazi

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Micromorphological Studies On Implantation Of Albino Rat Endometrium

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Received: 01 / 12 / 2020; Accepted: 31 / 12 / 2020

الملخص:

زرع البويضة في الرحم هو احدى الأحداث البيولوجية الأكثر إثارة للاهتمام، ودلائل اوليه للتفاعل البيولوجي للكيسة الجنينية مع الرحم أثناء حدوث الحمل. في هذه الدراسة اختيرت الفئران كحيوانات تجريبيّة بسبب; توافرها، وحجمها المناسب، وفترة حملها القصيرة، وكنماذج مقبولة لدراسة زرع البويضة بالرحم لأنها كانت تصنف المشيمة الناضجة لها تحت نفس النوع من المشيمة البشرية "نوع الهيموكورية" اي ان المشيمة معتمدة على تدفق الدم كما في الانسان . و كذلك لديها نفس الية انبات البويضة بالرحم والتي تدعى بالزرع الخلالي (interstitial implantation) كما هو الحال في الانسان . استخدمت لهذه الدراسة تسعة فئران بيضاء إناث عذراء ،تبلغ من العمر ثلاثة أشهر و كان وزنها ما يقرب من 200 غرام. كما تم تزاوج ست إناث من هذه الفئران (المجموعة 1) من قبل فئران ذكور من نفس السلالة، اما الثلاث المتبقية من الفئران لم يتم تزاوجها (المجموعة 2) . وقد أجريت هذه الدراسة لتوضيح مورفولوجيا (وصف مجهري للشكل) للتغيرات الطبيعية التي تحدث في الرحم أثناء زرع البويضة في الفترة المبكرة من الحمل بالتوازي مع وصف مجهري للرحم اثناء فترة عدم الحمل . مواقع زرع البويضة بالرحم كانت واضحة بشكل العيان كتورمات مثل الخرز. وكانت التغييرات الأكثر وضوحا كبيرة في المقاطع العرضية من بطانة الرحم في موقع بدء الزرع، في اليوم (7) dpc(يوم ما بعد الجماع)، حيث ان الخلايا السترومية الموجودة في بطانة الرحم(stromal cells) تم تكيفها لتشكل (decidualization) ، والتي قسمت بطانة الرحم إلى أربع مناطق رئيسية في كلا الجانبين ، أي ، (mesometrially) و (antimesometrially وكانت على النحو التالي: المنطقة الحاسمة الأولية(PDZ) , المنطقة الحاسمة الثانية (SDZ), منطقة الزرع (IZ)) و المنطقة الأساسية الغير متمايزة (UBZ). وعلاوة على ذلك، فإن توزيع الجليكوجين؛ المخاط متعدد السكريات المحايد والحمضي (neutral & acid mucopolysaccarides) الانزيمات الفوسفاتية القلوية والحامضية (alkaline & acid phosphatases) ؛ كانت متغير في مختلف المناطق ببطانة الرحم في اليوم (7 dpc)، وفقا لدرجة التلوين حيث تمت دراستها و مناقشتها.

الكلمات المفتاحية:

فئران، الزرع الخلالي، ديسيديوليزاتون، علم الأنسجة.

Abstract

Implantation is one of the most interesting biological events and marks the first biological interaction of the blastocyst with the uterus during the establishment of pregnancy. In the current study rats were used because of their availability, convenient size, short gestation period, and because their mature placenta is classified under the same type as the human placenta (Haemochorial type). They have the same mechanism of placental implantation documented for humans, i.e. interstitial implantation. Nine three months old albino virgin female rats were used for the study weighing 200 gram on average. Six females of albino rats (Group 1) were naturally mated by apparently normal male rats of the same strain. The remaining three females (Group 2) were not mated and as such used as control. This study was carried out to elucidate the normal morphological changes occurring in uterus during implantation and early pregnancy. Upon dissection, the implantation sites were apparent macroscopically as beads like swelling. The most significant changes were seen in the cross sections of the endometrium at day (7) dpc. They were evident at the initiation site of implantation. The stromal cells were modified to decidualization and divided into four main zones in both sides, i.e., mesometrially and antimesometrially; the primary decidual zone (PDZ), the secondry decidual zone (SDZ), the implantation zone (I.Z), and the undifferentiated basal zone (UBZ). Moreover, the distribution of glycogen, neutral and acid muco polysaccarides, alkaline and acid phosphatases were variable in different zones of the endometrium at day (7) dpc. Conclusion: One of the early events following implantation is stromal cell differentiation into a specialized type of cells termed the decidual cells (decidualization), which support embryo development. This process is initiated at the antimesometrial (AM) pole. Subsequently, decidualization proceeds mesometrially. The differentiation of the antimesometrial and mesometrial decidua proceeded radially parallel with the mitotic activity seen in stromal cells. This decidualization fully established on day 7 (dpc), dividing the endometrium into four main zones in both sides, i.e., mesometrially and antimesometrially.

Keywords: Rats, implantation, decidualization, histology.

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1. INTRODUCTION

The participation of the apical membranes of the uterine epithelial cells and the underlying stroma in the process of blastocyst adhesion, invasion and embedding makes them an interesting object in the study of changes occurring during early pregnancy. The endometrium is a complex multicellular tissue that undergoes dynamic remodeling to establish a microenvironment that is suitable for supporting a pregnancy[1]. Attainment of successful implantation depends upon the synchronized changes in the endometrium before and after the arrival of the blastocyst into the uterine cavity[2]. At the time of implantation, the trophoblast cells of the embryo adhere and then embed into the maternal endometrium and eventually establish placentation. At the same time, the endometrium undergoes decidualization, which is essential for successful pregnancy. Decidualization is defined as the post-ovulatory process of endometrial remodelling, which includes epithelioid transformation of the endometrial stromal cells into the highly specialized decidual cells with distinctive functions[3].Decidualized endomtrial stromal cells (ESCs) provide nutrition for the implanting blastocyst, and they are the main cell type in the deciduas[4]. Ultrastructural studies of the human decidual cells show the characteristics of the epithelioid cells: enlarged and rounded nuclei, increased numbers of nucleoli, dense membrane-bound secretory granules, cytoplasmic accumulation of glycogen and lipid droplets, and the expansion of the rough endoplasmic reticulum and Golgi complex[5].

In mammals, glycogen and other carbohydrates are very important for embryonic nutrition in the early stages of pregnancy and the majority of them are produced by uterine glandular cells[6].Issra Iz-Aldeen and Rasmi (2017) showed that glycogen in early stage of implantation was seen mainly in the uterine epithelium and anti-mesometriallys in subepithelial stroma[7]. The role of glycogen in rabbit implantation is important for later use in the implantationand placentaion processes, or as a reserve of easily available energy[8].Matthew Dean (2019) reported that in mated rats, the glycogen content of the endometrium increases again after implantation due to high levels of glycogen stored in the deciduas[9]. In addition to glycogen other factors have been evaluated in early pregnancy in different animals including the rat and mouse. Such factors include alkaline phosphatase activity in the uterus, which play a role in the nourishment and implantation of the blastocyst[10]. The aim of our study on the rats reproduction were mainly concentrating on description of the normal processes occurring in uterus during implantation and early pregnancy.

2. MATERIALS AND METHODS:

In the present work rats were selected to carry out the experiments because of their availability, convenient size, short gestation period, and their suitability to study implantation. Their mature placenta is classified as an haemochorial type, the same type of the human placenta. In addition, they have similar mechanism of implantation to that occurring human, i.e. interstitial implantation[11].

Nine three month-old albino virgin female rats weighing approximately 175-225g body weight were used for this study. They were maintained on a program of 14 hours light and 10 hours dark cycle. They were on concentrated ration pellets and tap water ad libitum.

Six females of albino rats (Group 1) were mated by normal male rats of the same strain. The morning of finding sperms in the vaginal smear was designated as the day one of pregnancy (figure 1). The sperms were fixed in 95% ethylalcohol for 15 minutes and stained by Haematoxylin and Eosin (H&E) stain.

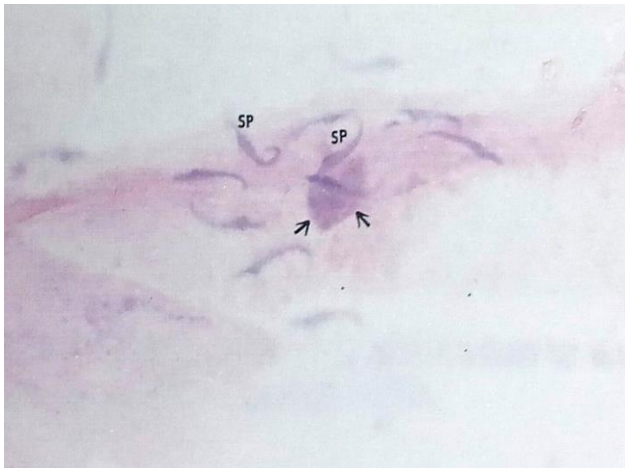


Figure 1: Showing the rat estrus phase of cycle, characterized by aggregate like masses of cellular material (arrow). Note sperms(SP) are appeared as period of sexual receptivity. Haematoxylin & Eosin stain. X40

The remaining three female rats (Group 2) were not mated, and were scarified to study the normal morphology and histochemistry of the non-pregnant uterus (N.P.U). Female rats of Group (1) were scarified on day 7 post coitus (dpc). Rats of both groups (1and 2) were anasthetized with ether, laparotomized, the uteri were removed and divided into segments. Segments of (group 1) containing the implantation sites were easily identified by their beaded appearance due to their endometrial swellings. Some segments were placed in 10% formal saline for 48 hours for histological and histochemical assessment. After a period of 48 hours fixation the delicate tissue was dehydrated solely using ascending grades of ethyalocohol. All samples were then cleared in xylene and embedded in high melting paraffin wax (57ºC). The blocks of group (1) were carefully oriented to have the cross sections to be cut from the implanted sites. Serial cross sections from 10% formal saline fixed samples, 5µm thickness, were cut and stained by the following stains: Haematoxylin and Eosin (H&E) stain were used for general morphological examination Periodic acid schiff (PAS) stain for localization of glycogen and neutral mucopolysacchrides; Alcian blue-PAS (AB/PAS) combined stains for the differentiation of neutral and acidic mucopolysacchrides[12]. Other segments of tissue were snap frozen using aerosol spray (-50ºC), which was particularly useful for attaching stored cork discs, bearing frozen tissue on to cryostat chunks. These unfixed frozen tissues were used for enzyme histochemistry. For alkaline and acid phosphatase enzymes: The optimal temperature of the cryostat used was -20ºC. Twelve (12µm) thick sections were cut from those unfixed frozen tissue samples. They were mounted on the slides in cryostat cabinet till the time of preparation of the substrate, which was done on the same day. Gomori's method for alkaline and acidphosphatase enzymes stains was followed12, and all stained slides were examined under the light microscope.

3. RESULTS:

The normal endometrium in the rats has multiplicity of constantly changing normal patterns that depend onthe nature and intensity of the ovarian hormone stimulation. Cross sections in the normal non pregnant uterine horns of female rats were found to consist of outer peritoneal and serosal investiture over thick smooth muscle myometrium arranged in distinctly oriented layers. Internally, the horns had two layers that were identified within the endometrium throughout this region. The lowermost endometrium was recognized as the sratum basalis and the overlying one was the stratum functionalis (figure 2a).

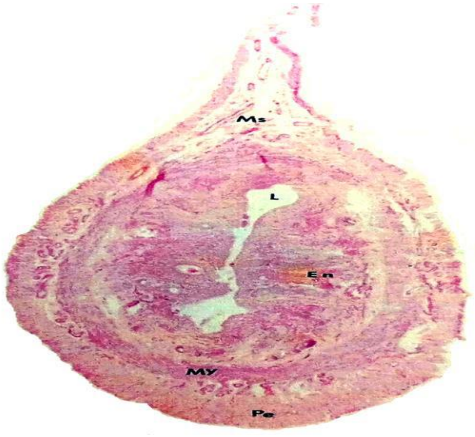


Figure 2a: Cross section in the normal uterine horns of female rats consisting of outer peritoneal and serosal investiture (pe), over a thick myometrium (My). Internally two layers of the endometrium (En) .PAS- Haematoxylin counter stain X10.

The appearance of the basalis was relatively constant throughout the estrus cycle, i.e., in all proestrus, estrus phases (proliferate endometrium), metaestrus, and diestrus phases (secretory endomerium), maintaining a weakly proliferate appearance. The stratum basalis was the zone with weakly proliferate glands having associated dense spindled stroma immediately adjacent to the myometrium. The remainder of the endometrium was the functionalis. This layer had been traditionally divided into two strata: the superficial compact layer, the stratum compactum and the deeper spongy layer, the stratum spongiosum. The glands and the surface epithelia of the functionalis were both composed of three different types of cells: secretory cells, basal proliferating cells and the ciliated cells (figure 2b).

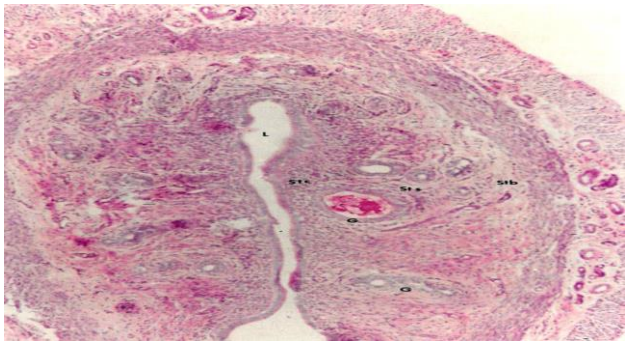


Figure 2b: Showing different strata of endometrium. Stratum basalis (Stb), stratum compactum (Stc) and stratum spongiosum (Sts). Note secretory glands (G). PAS- Haematoxylin counter stain X40.

Generally, the glands in the proliferate endometrium appeared to be similar to undifferentiated basalis. However, the appearance of the glands in the secretory endometrium had tortuous outline and were characteristically fairly distended and filled with glandular secretion. They were mainly lined by non-vacuolated secretory cells (figure 2c).

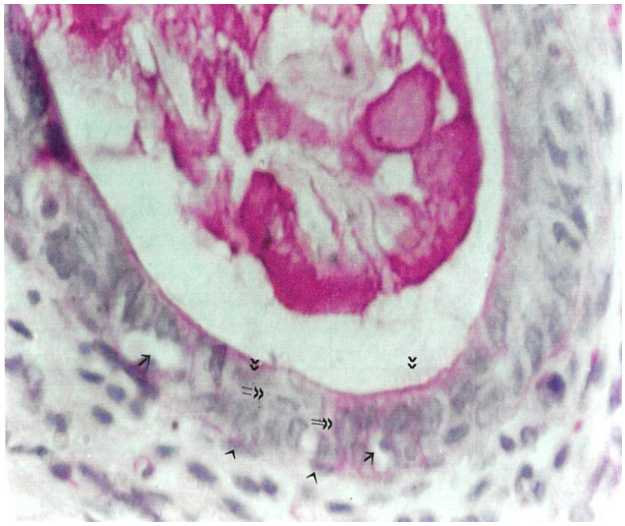


Figure 2c: Showing the three different types of cells forming the wall of glands. Secretory cells (arrow), basal cells (arrow head) and ciliated cells (double arrow head). PAS- Haematoxylin counter stain X100.

The endometrial stromal cells were the predominant cellular component of the stroma, and it's appearance varied greatly with the stages of the estrus cycle. In the proliferate endomtrium, the stromal cells were fibroblast like, and appeared to be of small spindle shape, while in the secretory endomtrium, they were large with vesicular nuclei and their cytoplasm was abundant and stained pale stained (figure 2d).

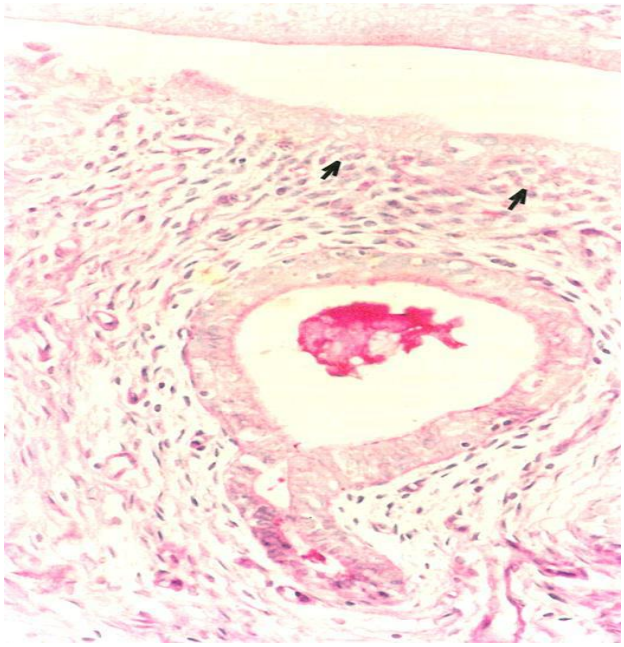


Figure 2d: Condensation of large stromal cells, with their vesicular nuclei (arrow) of endometrium of non-pregnant rat PAS- Haematoxylin counter stain X40.

The localization of glycogen and neutral mucopolysacchrides detected by the amount of PAS materials was seen as sparsely distributed within the rat endometrium during the proliferate period. There were traces of coarse red granules situated basally or on the apical portion of the glandular and epithelial cells. In the endometrium, during the secretory period, the epithelial cells especially the glandular cells, were loaded with +ve material in particular the apical portion of the cytoplasm. Similarly, the gland lumen was filled with this material. The extracellular spaces reacted positively. Strong reaction have also been found within the basement membranes of glandular and surface epithelial and often within the wall of the blood vessels (figure 2 a,b & c). There was a very weak reaction in the stratum basalis of both proliferate and secretory endometrium. To study the distribution of the mucopolysaccharides (MPS) within the layers of the endometrium in the non-pregnant uterus of the rat the Alcian blue-PAS (AB/PAS) technique was used.This technique was selected to distinguish acid mucopolysaccharides (staining blue) from neutral mucopolysaccharides (staining red).

The stratum compactum of both the proliferate and secretory endometrium were expressing a bluish coloration indicating the presence of acid MPS, while the stratum spongiosum and the rest of the endometrium showed no reaction to this stain. On the other hand, the glandular lumen was filled with red and bluish colored secretion indicating the presence of both acid and neutral MPS (figure 3).

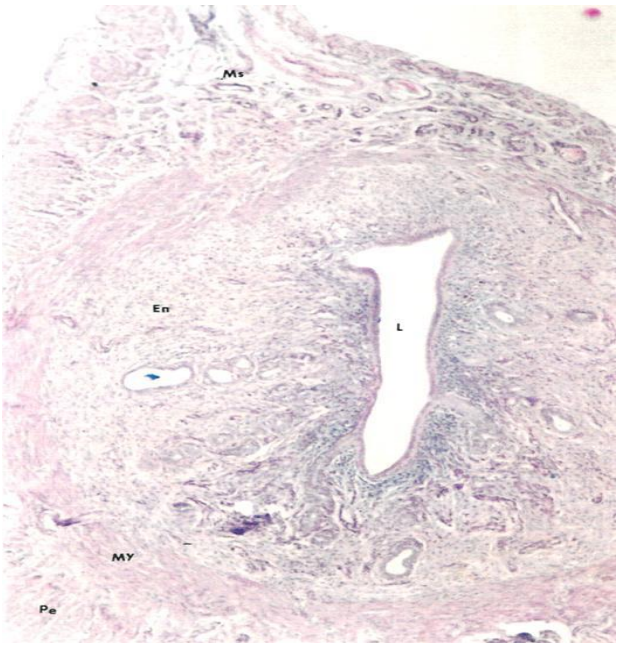


Figure 3: Showing distribution of MPS, acidic (blue color) and nuteral (red color) within the layers of non-pregnant uterus. Alcian blue-PAS stain X40.

The reaction of Alkaline phosphatase (ALP) in the non - pregnant uterus was variable according to its appearance within the layers of the endometrium at different of estrus cycle. In both, the endomtriumproliferate andsecratory phases, the epithelial and capillaries were rich in ALP. Gland cells and luminal epithelial surface cells expressed the enzyme in all their structures, in the nuclei and cytoplasm, and their basement membranes (figure 4a). The enzyme expression in the stroma was almost negative in both the proliferate and secretory endometrium (figure 4b).

The distribution of acid phosphatase(ACP) within the endometrium was very weak, and no reaction products were seen in either the proliferate and secretory phases. In some tissue sections, there was very weak reaction for ACP within the glandular and luminal epithelial surface cells and in the wall of blood vessels of both the secretory and proliferate endometrium (Figure 4 c).No reaction productswereconsistently observed in any of the stromal cells in either phases of estrus cycle.

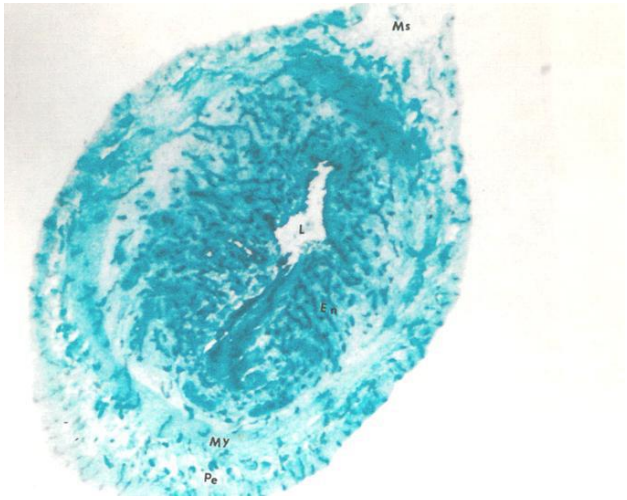


Figure 4a: Demonstrated the distribution of alkaline phosphatase enzyme within the wall of non pregnant uterus. Gomeri's method for AlP. Stain.X10

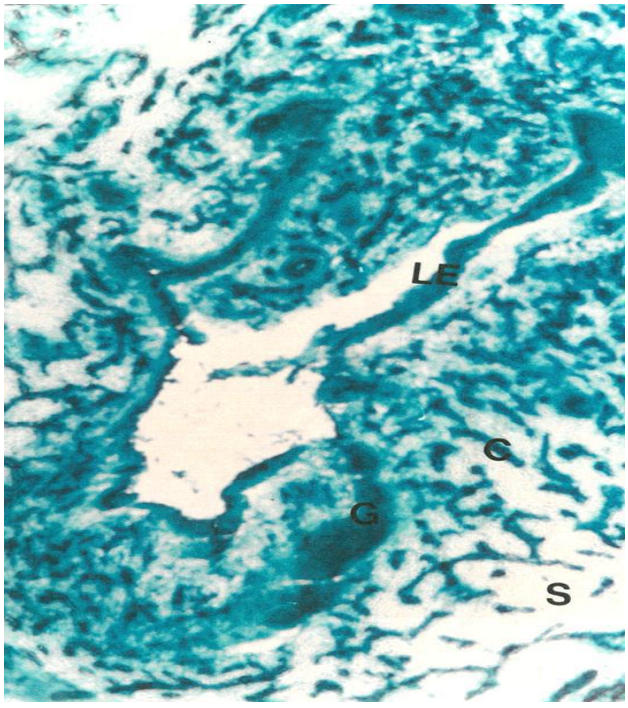


Figure 4b: Strong reaction for AlP (black color) restricted in luminal epithelium (LE), endometrial glands (G) and blood capillaries(C),while the rest of endometrium do not react. stroma (S). Gomeri's method for AlP. Stain.X40

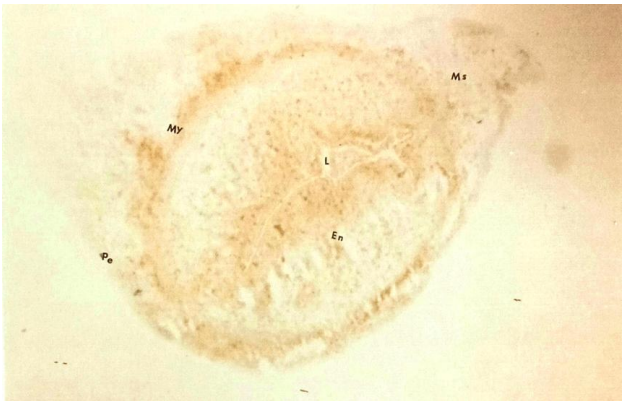


Figure 4 c: Showing the distribution of acid phosphatase enzyme within the wall of non-pregnant uterus. Gomeri's method for AcP. Stain.X10

Following implantation, and the initiation of the primary decidual response in group 1 of female rats that were scarified on day 7(dpc), the uterus at day 7(dpc) underwent noticeable modification in the endometrium leading to restructuring of the implantation region. The implantation sites were evenly distributed in each uterine horn, and the number of sites per horn ranged from 3 to 5. At this selected day of pregnancy (day 7) the embryos were oriented and resided in antimesometrial positions of the endometrium of the uterus. The implantation sites were macroscopically apparent as beads like swelling, which could be seen along the full length of uterus. On the outside of these swellings the uterine lumens were small and positioned eccentrically close to the mesometrial side of the uterus. Towards the center of the swelling, the lumens wereantimesometrially enlarged, forming a crypt in the middle of the decidual mass.

4. HISTOLOGICAL FINDINGS:

Decidualization produces two uniquely different cell populations of the stromal cells, depending on the relative position of the cells to the implantation site. The initial site of stromal cells modification for decidualization was in the antimesometrial side of the endometrium to from the antimesometrial decidua which would be the forerunner of the future of decidua capsularis. Subsequently, decidualization proceed mesometrially, where stromal cells from the mesometrial decidua, which would be the forerunner of decidua basalis. The differentiation of the antimesometrial and mesometrial decidua were proceeding radially parallel with the mitotic activity seen in the stromal cells (figure 5).

In the tissue cross section (Plate No. 1), the endometrium at day 7(dpc) could be divided into four main zones in both sides (mesometrially and antimesometrially):

1. The primary decidual zone (PDZ).
2. The secondry decidual zone (SDZ).
3. The implantation zone (I.Z).
4. The undifferentiated basal zone (UBZ).

The PDZ was a cup-shaped region of transformed fibroblast like stromal cells surrounding the blastocyst and luminal epithelium.

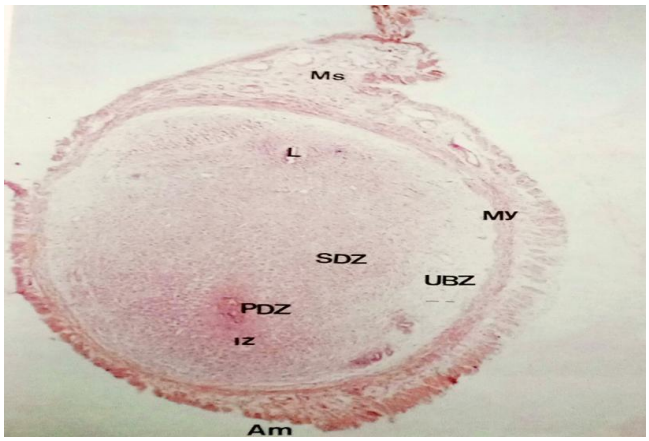


Figure 5: Cross section of rat uterus at day (7) dpc, showing the decidal reaction leading to enlargement of uterus. Note, the endometrium divided into four main zones. Haematoxylin & eosin stain. X10.

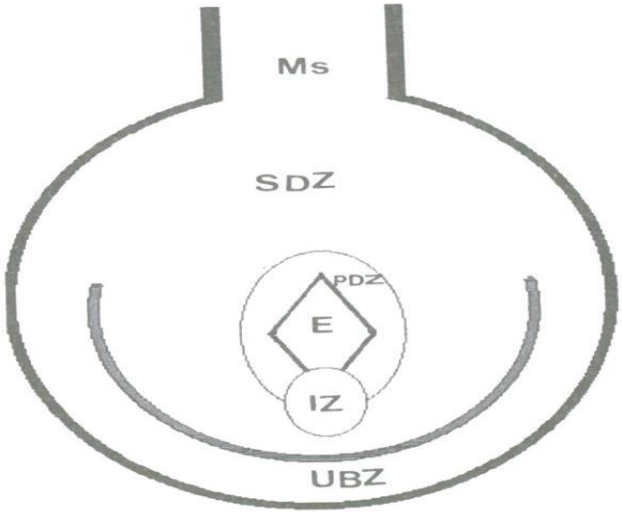


Plate 1: The endometrium at day (7) dpc, could be divided into four main zones: The primary decidual zone (PDZ).The secondry decidual zone (SDZ).The implantation zone (I.Z). and The undifferentiated basal zone (UBZ).

On the morning of day 7(dpc), the PDZ was merely a cylindrical group of large closely packed decidual cells that extended mesometrially above the embryo and antimesometrially well below the embryo (figure 6 a). The full extent of the PDZ was on both sides of the embryo, i.e., the right and left side.Initially the most obvious cellular changes were seen in the antimesometrial side of the endometrium. In this region, extensive proliferate active stromal cells and densely formed packed mass of large and often binucleated cells were seen(figure 6b). The SDZ was situated inbetween the PDZ and the UBZ. Ithad a spongy appearance, and it occupied most of the area of the endometrium, forming a circle around the PDZ. The demarcation between the PDZ and SDZ could be outlined by the presence of the blood vessels at the peripheral boundry of the PDZ(figure 6c).

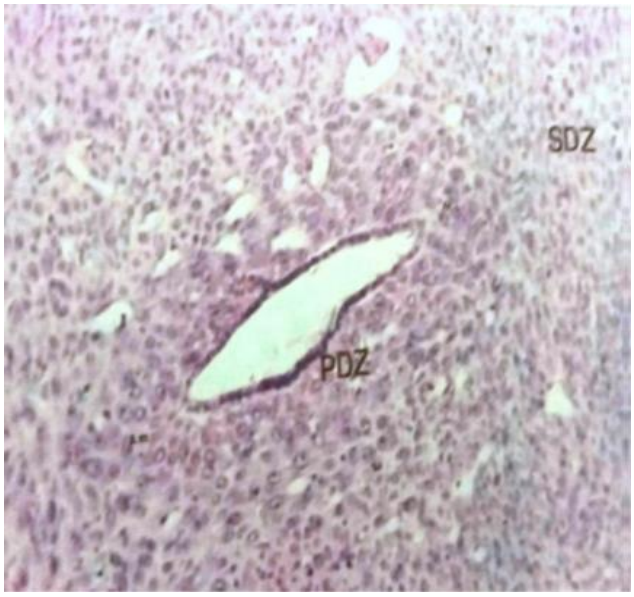


Figure 6 a: Primary decidual zone (PDZ) was a zone of compact cells extended both mesometrially and antimesometrially. H&E stain X20

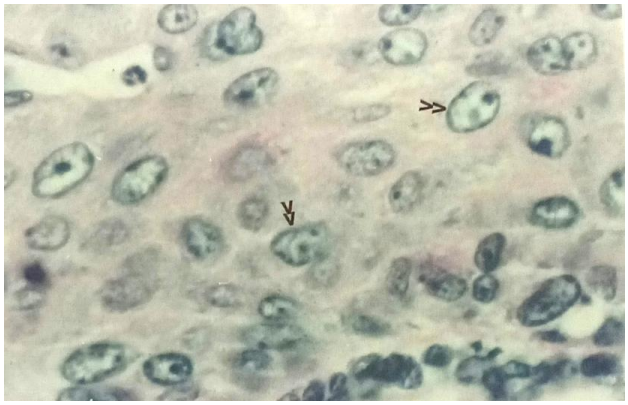


Figure 6 b: Binucleated cells (double arrow head) were seen in PDZ. H&E stain X100

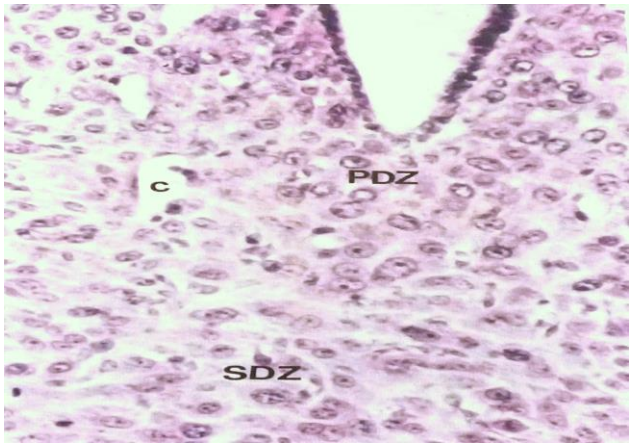


Figure 6 c: Secondary decidual zone (SDZ) have spongy appearance. Note, demarcation between the PDZ and SDZ, could be outlined by presence of the blood capillaries (C) H&E stain X40

The demarcation between the SDZ and the UBZ was revealed when the undifferentiated stromal cells and glands start to appear in the field (figure 7). Morphologically, the cells of SDZ were similar to the cells of the PDZ, but they were less packed leaving intercellular spaces between them. The incidence of mitotic activity was considerably higher in this zone than in the PDZ. The Implantation Zone (I.Z) was a small zone located antimesometrial to the embryo where the epithelium was denuded and the surrounding decidual cells in close vicinity to the embryo were degenerated. This zone was recognized by the presence of wide intercellular spaces (figure 8a) leaving the decidual cells disarrayed. The cells of the I.Z zone were small polygonal cells with many processes containing small round nuclei. The nuclei became larger and ovoid in shape when cells were in close vicinity to the antimesometrial side of the SDZ. The undifferentiating basal Zone (UBZ) was a narrow band of tissue extending about 3/4 of the way around the circumference of the endometrium. It was usually widest antimesometrially, separating the decidual tissue of the SDZ from the inner circular layer of the myometrium (figure 8b).The cells of the UBZ were undifferentiated flattened fibroblast like stromal cells.

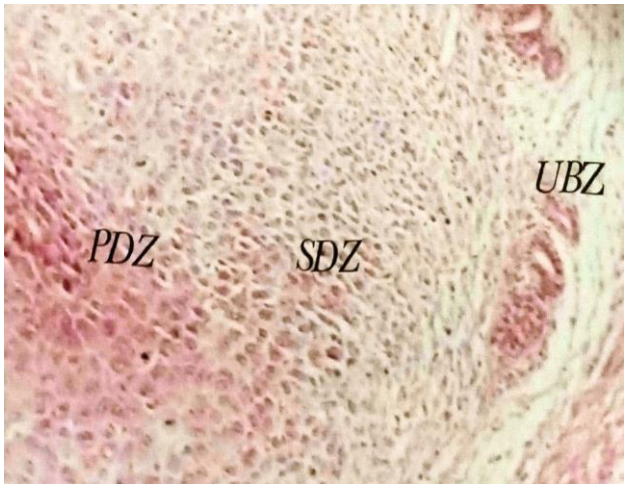


Figure 7: Cross section in the implantation site of rat endometium at day (7) , showing the three different zones PDZ, SDZ & UBZ. H&E stain X20

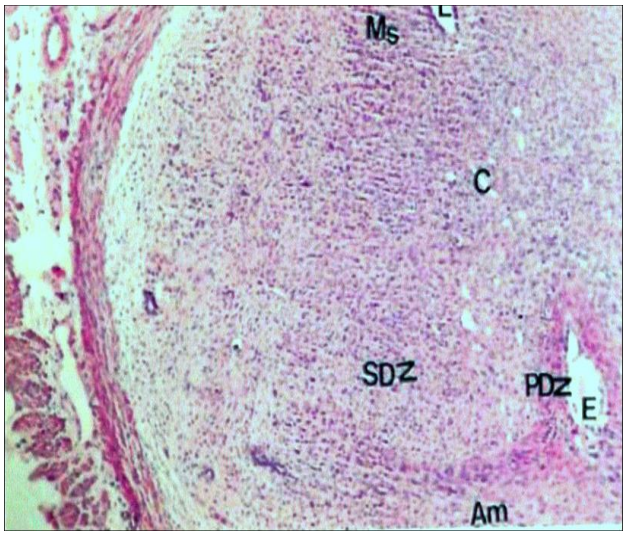


Figure 8 a: Showing implantation zone (I Z) was small zone located antimesometrial to the embryo (E) where the epithelium were degenerated. Note PDZ has strongly PAS +ve materials. PAS stain X20

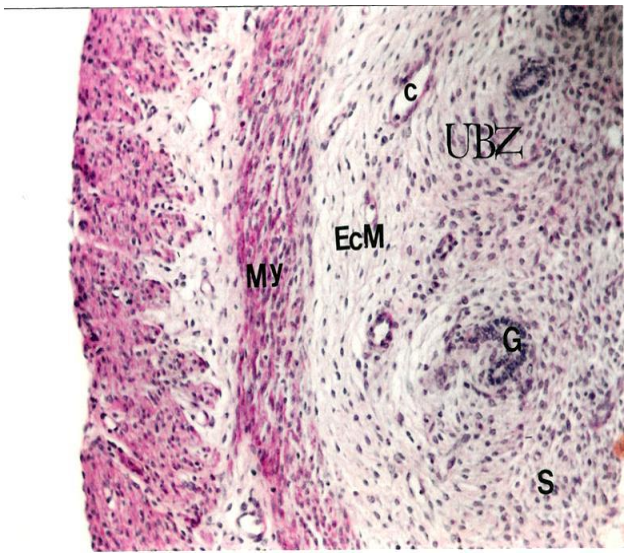


Figure 8 b: Showing the undifferentiating basal Zone (UBZ) was a narrow band of tissue extends around the circumference of the endometrium without PAS reaction except some faint PAS +ve reaction in the extracellular spaces (EcM). PAS stain X20

5. HISTOCHEMICAL FINDINGS:

Distribution of PAS on the zones of the rat endometium at day 7 (dpc)was variable. Strong PAS reaction was found in the PDZ region. The cells of this zone were loaded with magenta color granules. These granules representedcellular positivity of glycogenic material that confirmed the presence of the glycogenic mucopolysaccharides (MPS-figure 8a). Faintly positive PAS reaction appearedat the lateral side of the implanted blastocyst towards the antimesometrium position, in the cells of the SDZ. This was in addition to the presence ofsporadicallyseen cellsfilled with glycogenic granules, appearing here and there among the cells of the SDZ. No positive reaction was seen within the cells of the UBZ, but some faint PAS +ve reactions were seen in the extra cellular spaces. Glands which were shifted to this area after the growth of decidual cells showed no reaction. While, strong +vePAS reaction was noted in the walls of the blood vessels (figure 8 b). To confirm these results positive reaction for PAS stain was obtained in the smooth muscle fibers of the myometrium of the same section.

Section of rat endometrium taken on day 7(dpc) stained with combined alcian blue/Pas stain (Ab/PAS) showed a degree of coloration indicating variable distribution of the MPS(plate 2) i.e., Strong degree of magenta coloration appeared in the PDZ surrounding the implanted blastocyst, indicating the presence of neutral MPS in this zone. The SDZ showed acidic MPs indicated by the appearance of bluish coloration that were spread throughout the whole zone (figure 9a). Faint magenta coloration appeared in the extracellular spaces of the UBZ suggesting the presence of weak reaction of the neutral MPS.Glands of this zone showed neither acidic nor neutral reactions. On the other hand, the blood vessels reacted for neutral MPS suggested by the presence of magenta color within their walls (figure 9 b).

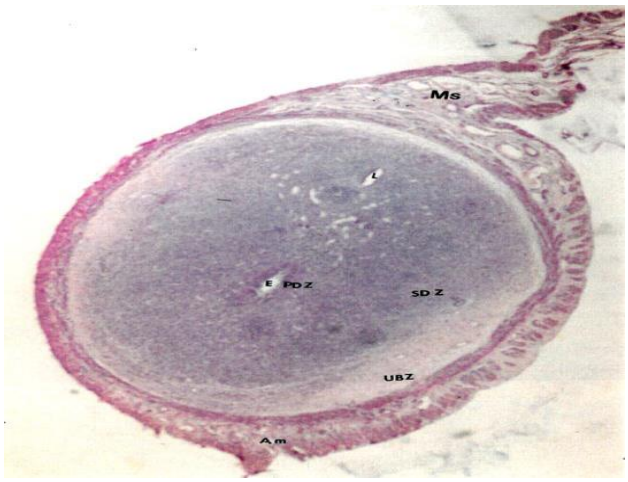


Figure 9 a: Showing distribution of the MPS were variable according to the degree of coloration. PDZ= Strong neutral MPs magenta coloration, SDZ= Acidic MPs bluish coloration, and faint reaction of the neutral MPS in UBZ. Ab /PAS stain. X10

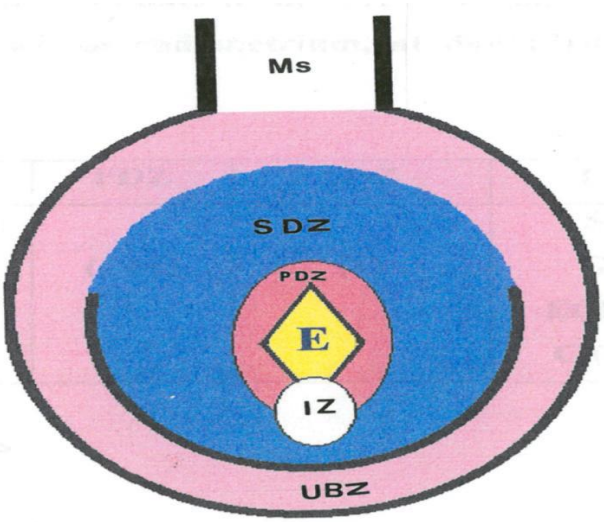


Plate 2: Distribution of neutral and acidic MPS were variable according to the degree of coloration. Strong neutral MPS = Magenta color, Faint neutral MPS= Pink color, Acidic MPS = Blue color, and white color=No reaction.

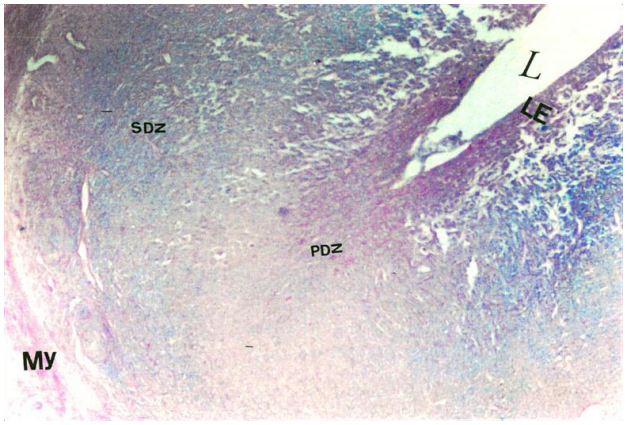


Figure 9 b: High magnification of rat endomtrium at day (7) dpc, showing the distribution of MPS in different zones. Ab /PAS stain. X40

The reaction of ALP in the rat endometrium sections at day 7 (dpc) varied from one zone to the other. Very intense and strong reaction was seen in the PDZ, surrounding the implanted blastocyst indicated by the presence of black coloration. However, the implantation zone immediately outside the embryo was less reactive (figure 10 a). In the SDZ, the intensity of reaction was very strongantimesometrially, and the same degree of reaction was seen in its blood vessels. The remaining parts of the mesometrial SDZ were showing weak to no reaction. In the UBZ there was no reaction for ALP in both undifferentiated stromal and glandular cells, while a strong intense color was seen in the wall of the blood vessels and in their endothelial lining cells (figure 10 b).

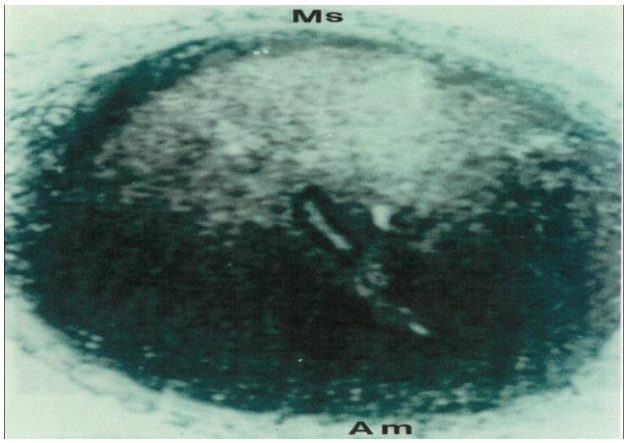


Figure 10 a: The reaction of (AI.P) in the rat endometrium sections at day (7) dpc, were variable in different zones. Very intense and strong reaction was seen in the PDZ. Gomeri's method for AlP. Stain.X10

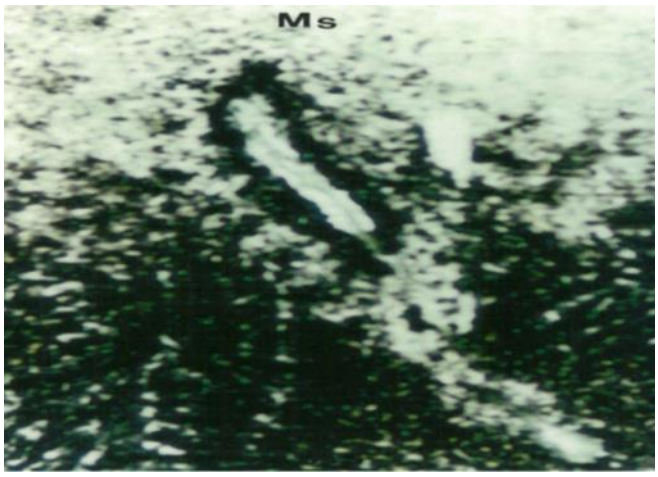


Figure 10 b: Showing no reaction for (AI.P) in the UBZ, while very intense and strong reaction was seen in the PDZ & SDZ Gomeri's method for AlP. Stain.X40

The reaction for ACP at day 7 varied with endometrial zone type. There was no reaction for ACP in the PDZ and SDZ of both sides (mesometrially and antimesometrially). However, there was a weak to moderate reaction in the I.Z. indicated by the presence of brown coloration within this zone.In the UBZ, the glands, the stromal cells and the extracellular matrix had no reaction for ACP. In the intact luminal epithelium in both sides, mesometrial and antimesometrial, there was moderate to strong (Ac.P.) reaction was showed in all sections (figure11).

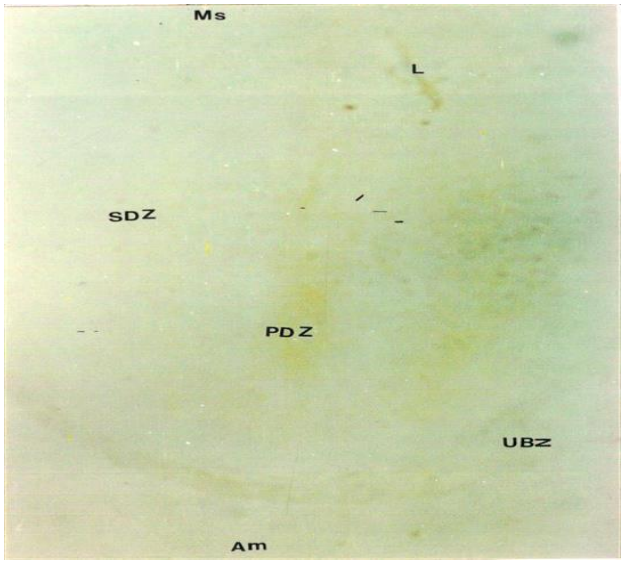


Figure 11: Showing the reaction of (Ac.P), also was variable in different zones of the endometrium at day (7).There was no reaction for (Ac.P) in the PDZ, SDZ and in UBZ. Moderate to strong Ac.P seen in the luminal epithelium (L). Gomeri's method for AcP. Stain.X10

6. DISCUSSION:

The present study has confirmed the results of other studies[13,14,15,16]. It has extended our knowledge of the morphological effect induced by the presence of implanted blastocyst on the uterine tissue during early pregnancy. Furthermore, it has demonstrated that there are striking changes during cellular the development and differentiation of stromal cells of the endometrium to decidual tissue due to the presence of implanted blastocyst. The regional variations of different decidual zones seen in the present study revealed highlevels of structural organization characterizing the programmed decidual response in the pregnant uteri of female rats. The results will be first discussed in relation to other studies on the effect of implantation on the uterine tissue; second in relation to studies on the distribution of glycogen, mucopolyssacarides and phosphatase enzymes during the early period of gestation. The discussion of the results of present work will be built according to the available literature on normal pregnancy in the rat[14,17]. These studies have indicated that the uterine environment and the implantation reaction, for the presence of blastocysts at day 7 dpc, have offered optimum conditions for development. One of the prominent changes noted in the current study was the presence of decidual reaction. Welsh and Enders (1985) have pointed out that further expansion of the conceptus is closely tied to changes in decidua[14]. By comparing the results of day 7 dpc, it was apparent that the conceptus and the endometrium underwent marked changes reflecting a normal sequence of events seen in early normal pregnancy of the rat[14,15]. Light microscopic examination of the decidualization reported here in confirms the high levels of structural complexity and regional variation that characterize the decidual reaction.

As shown in this study and in previous studies on implantation in rats[18], in rabbit7 and in mice[19], the endometrial stromal cells between the luminal epithelium and the first layer of blood vessels at the antimesometrial side of the uterus was transformed into a primary decidual zone (PDZ). Among the features of this zone that were demonstrated in the present work was the tightly packing of their cells and the absence of blood

capillaries. The absence of capillaries from PDZ was demonstrated by using the vascular corrosion casting and scanning electron microscopy technique[20]. Despite its apparent avascularity, it was shown by using intravenously administrated tracers that this zone was permeable to tracer of molecular masses of 45 KDa or less. These tracers penetrated the intercellular space of PDZ within 10 minutes after administration[18]. Such finding suggested that this zone may restrict the passage of cells nutrients and maternal immunoglobulin, thus provides immunological protection for the embryo and prevents micro-organisms from reaching the rat embryo before it develops its own protective layers at the early stages of pregnancy. Several kinds of intercellular contacts between decidual cells in the PDZ have been described. These include tight junctions[17] and gap junctions that are responsible for intercellular communication between decidual cells in mouse[21]; in hamster [22]and in human[23]. In addition extensive inter-digitation of plasma membrane between decidual cells forming a complex membranous labyrinth together with numerous contact between decidual cells of this zone, including adherens and gap junctions have been described in developing deciduoma of the pseudo - pregnant rat[13]. These findings suggested a barrier function of PDZ, however, decidual cells could play a major role in maintaining coherence and structural support for this interdigitation in which collagen fibrils are present in very small numbers. Beaulaton and Lockshin (1982) suggested that communicating junctions serve as transmitting channels between cells that appear to interpret the information relating to their position, supposedly, by shifts of inorganic ions or cyclic nucleotides[24]. In this manner groups of cells are able to coordinate their activity. The lack of blood vessels implies a reduction of blood flow to the implantation site, which seems paradoxical in view of the growth of the developing embryo. This suggests that the embryo at day 7 of pregnancy might still depend on histotrophic source of nutrition[25,26]. Such aconclusion was in accordance with the accumulation of glycogen and neutral mucopolysaccharides in the PDZ.In contrast to that the equivalent subepithelial region of non-pregnant rat endometrium “Stratum compactum” is known to demonstrate acid mucopolysaccharides constituents only. Similarly, the SDZ of day 7 dpc was showing positive reaction for acid MPS.Thus PDZ may serve as a temporary barrier preventing maternal micro-organism and immuno-competent cells from reaching the rat embryo before it has developed its own protective layers. Possible function for PDZ as a protection for maternal tissue from further invasion of trophoblastswere suggested[27].Yuan et al.,(2019)reported that in micethestromal cells transform into epithelial-like cells to form the avascular primary decidual zone (PDZ) around the implantation chamber (crypt)[19]. The PDZ creates a permeability barrier around the crypt restricting immune cells and harmful agents from maternal circulation to protect embryonic health. The morphological and the histochemical assessments of day 7 dpc have revealed that the transformation of the SDZ was gradual and its morphological and histochemical changes were conditioned by its distance from the luminal epithelium and the trpohectoderm of the implanted blastocyst, which have come into direct contact with the PDZ17. Among the points noticed in this study was the reduction in size and the decrease in activity of the endometrial glands. Their glandular orifices totally disappeared, and they were displaced toward the myometrium in the undifferentiated basal zone "UBZ".

Bucci and Murphy (1995) reported that the glandular orifices had disappeared from the endometrial surface at day 5 dpc in the rat. They attributed that to the apparent overgrowth of the surface epithelium. At this stage of pregnancy the decidual tissue is not yet formed[28]. It was noticed in the present study that there was pronounced rearrangement of the endometrial tissue, which occurred at day 7 dpc along with the uterine luminal epithelium disappearance at the antimesometrial side.At this stage of pregnancy, the antimesometrial decidual tissue reached its maximum thickness, and that was the main reason of pushing the glands towards the myometrium, in the UBZ. Therefore, the antimesometrial side of the uterus, where the glands were located was clearly identifiable, and was differentiated from the mesometrial side, where the glands were absent.

The appearance and distribution of glycogen in the rat endometrium reported in the present study was essentially the same as that previously described[13,14]. In addition, similar distribution was reported in the pregnant hamester[22].The rat and hamster conceptus during early pregnancy still nutritionally dependent upon glycogen as a source of histotrophic nutrition. Matthew Dean (2019)suggested that the embryo was probably supported by circulating blood glucose. Endometrial glycogen concentrations are correlated with fertility in humans, indicating that glycogen is an essential source of glucose during early pregnancy[9]. In mated rats, the glycogen content of the endometrium increases again after implantation due to the high levels of glycogen stored in the decidua. The earlier accumulation of glycogen at day 7 dpc appeared in the PDZ. In this zone, the decidual cells containing glycogen and neutral MPS, appear to be very active metabolically.If, as seems likely, the decidual cells synthesize substances of nutritive value to the conceptus, these substances might enter the embryo in two ways. Firstly, they could be acquired as the trophoblast phagocytosed glycogen containing decidual cells. Hence, it seems reasonable to assume that these cellular products are broken down by lysosomal hydrolytic enzymes in the trophoblast cells. Secondly, substances from the PDZ cells could reach the blastocyst by diffusion. It is interesting that the trophoblast cells are joined together by tight junctions, which are thought to allow passage of quite large molecules between cells[29].

Alkaline phosphatase is an ancient enzyme that was thought to have insignificant physiological roles since it hydrolyses phosphate esters at high alkaline pH[30]. The present study have demonstrated the presence of a relationship between decidualization and alkaline phosphatase activity. Similar finding was reported in rat[31].Winkelmann and Sponitz (1997) concluded that ALP activity is an indicator for decidualization.This enzyme plays a very important role in early phases of implantation[32].The current study showed that alkaline phosphatase activity was noticed to increase in the uterine epithelium and in the PDZ during early implantation. On the other hand, luminal epithelial cells showed apical plasma membranealterations such as polarity changes and flattening of microvilli. These changes occurred with the reorganization ofapical molecules during epithelial cell surface preparation for blastocyst attachment in a variety of species such as rat[28],human[33] and mouse[34]. In contrast to the presence of alkaline phosphatase activity in the decidual tissue of animals, the present results have indicated the presence of faint or no reaction products for acid phosphatase activity. Acid phosphates were found among the lysoscomal enzymes to reflect the activity of lysosomal hydrolases in general.

7. CONCLUSION:

This article outlined the results with discussion of the main events of implantation and decidualization in rats. One of the early events following implantation is stromal cell differentiation into a specialized type of cells termed the decidual cells (decidualization), which support embryo development. This process is initiated at the antimesometrial (AM) pole with the transformation of stromal cells into epithelial-like cells (endometrial decidual cells) surrounding the implantation site that houses the blastocyst. Subsequently, decidualization proceeds mesometrially. The differentiation of the antimesometrial and mesometrial decidua proceeded radially parallel with the mitotic activity seen in stromal cells. This decidualization begins to form from day 6 (dpc), and becomes fully established on day 7 (dpc),dividing the endometrium into four main zones in both sides, i.e., mesometrially and antimesometrially: the primary decidual zone (PDZ), the secondry decidual zone (SDZ), the implantation zone (I.Z),and the undifferentiated basal zone (UBZ). The PDZ was avascular group of large closely packed decidual cells thought to function as a transient permeable barrier to protect the embryo from harmful agents. Histochemically PDZ was shown to contain glycogen andneutral mucopolysaccarides, and was shown to react positively for alkaline phosphatase, clear evidence that it is an immediate source of nutrition for the embryo. The SDZ wasa vascular area with loosely packed decidual cells forming a circle around the PDZ.Organization and histochemical reaction of this zone include faint positive reaction with PAS, strong positive reaction with acid MPS, sporadic cells filled with glycogen, an intense reaction for ALP and a weak to no reaction for ACP, suggesting that these changes were conditioned by their distance from implanted blastocyst and luminal epithelium. The UBZ was a narrow band of tissue separating the SDZ from the myometrium. The cells of thiszone were undifferentiated flattened fibroblast like stromal cells with faintpositive reaction for PAS and neutral MPS, no reaction for either ALP and ACP and it was occupying the remaining basal segment of the endometrial glands.It was suggested that this zone would participated in repopulation of the endometrial tissues during its subsequent regeneration.

8. RECOMMENDATION:

Further works will be needed to explore the ultrastructures using scanning and transmission electron microscope todemonstrate the process of invasion of blastocyst to maternal tissue until placenta formation.

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