

## Relationship of Granulated Metrial Gland cells to the trophoblastic barrier of mice and human placenta

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### ABSTRACT

**Background:** A study was performed on the presence of Granulated Metrial Gland (GMG) cells in mice uteri, similarly in human, the presence of human Decidual Granular Leukocytes (DGLs) in aborted placental samples was also studied.

**Aim of the study:** to demonstrate further evidence which may lead to the suggestion that the granulated metrial gland (GMG) cells are specialized immune cells which are involved in inhibition of the rejection reaction of the mother to her foetus as an allograft in pregnant mice uterus and it's relation to the decidual granular leukocytes (DGLs) in human pregnant mothers during early pregnancy.

**Material and Method:** Tissue samples of uterus were taken from mice on each of days (6, 10, 12, 14 and 16) of pregnancy, also human aborted placental tissue samples were taken at 2, 3 & 5 months. All samples were prepared by using routine histological techniques.

**Results:** Granulated cells were found in small numbers randomly distributed through out the endometrium on day 6 of pregnancy with a subsequent loss from areas of the antimesometrial and lateral decidua but increase dramatically in number in the developing decidua basalis sharing an intimate association with fibroblast-like stromal cells. Regarding human aborted placental tissue samples, similar to GMG cells, human Decidual Granular Leukocytes (DGLs) are the dominant cell population in 2 months aged deciduas, while in 3 and 5 months aged placenta, the placenta appeared with reduced populations and distribution of DGLs, they looked unhealthy. The loss of (GMG) cells from the implantation sites is accounted for either by degeneration in situ or by migration via vascular channels to the blood vessels of maternal placenta which suggested that it could be of functional significance.

**Conclusion:** Our results indicate that DGLs probably protect the materno-foetal unit from other effects causing disorders to the placental development, and it may play a role in the pathogenesis of idiopathic repetitive abortion.

العلاقة بين حبيبات خلايا غدة الأرومة الغاذية الرحمية لحاجز المشيمة للفئران والمشيمة البشرية

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**المقدمة:** دراسة أجريت على الخلايا الحبيبية لغدة المتريال لأنثى الفأر الحامل في الأيام ٦ و ١٠ و ١٢ و ١٤ و ١٦ من الحمل، وكذلك أجريت دراسة مماثلة للخلايا الحبيبية البيضاء للساقط القاعدي و لمشيمة أنساء الحوامل في الأشهر ٢ و ٣ و ٥ بعد الإجهاض.

**المواد وطرق العمل:** تم أخذ عينات الأنسجة من الرحم من الفئران في كل أيام (١٤، ١٠، ١٢، ١٤، ١٦) من الحمل، اتخذت إحباط عينات أنسجة المشيمة البشرية أيضا في ٢، ٣ و ٥ أشهر أعدت جميع العينات وباستخدام التقنيات النسيجية الروتينية.

**النتائج:** بينت الدراسة بأن عدد قليل من الخلايا الحبيبية ظهرت في رحم أنثى الفأر الحامل في أليوم السادس من الحمل وبدأت بزيادة ملحوظة مع تطور الساقط القاعدي، وأظهرت علاقة متينة مع الخلايا المكونة للألياف.

أما بالنسبة الى الخلايا الحبيبية البيضاء لمشيمة أنساء الحوامل المشابهة للخلايا الحبيبية لغدة المتريال لأنثى الفأر الحامل، وجدت هذه الخلايا بشكل سائد في المشيمة للشهر الثاني من الحمل. بينما أخذ العدد بالتناقص في الشهر الثالث والخامس بعد الإجهاض وكذلك ظهرت المشيمة متخلفة صحيا.

**المناقشة:** فقدان الخلايا وقتها من مكان تطورها في غده المتريال في رحم أنثى الفأر الحامل أثناء فترة الحمل ربما يرجع الى هجرة هذه الخلايا بواسطة الأوعية الدموية خاصة وأنها شوهدت في هذه بعض الأوعية المتصلة بالمشيمة إذ ربما تقوم بعمل وظيفي معين في المشيمة خاصة وان المشيمة هي الرابط الرئيسي بين الأم والجنين، ربما يكون هذا العمل هو حماية لأنسجه الأم والجنين من أي تفاعلات مضادة.

**الخلفية:** دراسة أجريت على الخلايا الحبيبية لغدة المتريال لأنثى الفأر الحامل في الأيام ٦ و ١٠ و ١٢ و ١٤ و ١٦ من الحمل، وكذلك أجريت دراسة مماثلة للخلايا الحبيبية البيضاء للساقط القاعدي و لمشيمة أنساء الحوامل في الأشهر ٢ و ٣ و ٥ بعد الإجهاض.

**هدف الدراسة:** الهدف من هذه الدراسة هو إظهار أي أدلة أخرى مما قد يؤدي إلى القول بأن خلايا الغدة الرحمية المحببة (GMG) هي الخلايا المناعية المتخصصة التي تشارك في تثبيط رد فعل الرفض من الأم إلى جنينها بمثابة طعم خيفي في رحم الفئران الحوامل وكذلك متابعة هذه الخلايا

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في المشيمة البشرية لغرض إثبات بأنها تقوم بنفس العمل والوظيفة التي تؤديها في مشيمة الفئران وعلاقتها بخلايا الكريات البيض ساقطي الحبيبية (DGLS) في الأمهات الحوامل في الإنسان أثناء الحمل المبكر.

*الاستنتاجات:* الخلايا القاتلة الطبيعية (NK) هي نوع من خلايا الجهاز المناعي التي تحتوي على حبيبات مع المواد الكيميائية وهي ربما تكون القامع الطبيعي لحماية التفاعل بين انسججه الأم و الجنين من الآثار الأخرى التي تسبب اضطرابات في تنمية المشيمة. وقد تجلى النشاط المشط للمناعة في الأشهر الثلاثة الأولى من الحمل في مشيمة النساء الحوامل في الإنسان والتي من الممكن أن تلعب دورا في الوقاية من هجوم مناعي خيفي من قبل الأمهات على الجنين وبالتالي منع الإجهاض العفوي. البحوث التي أجريت مؤخرا تسلط الضوء على حقيقة أن الخلايا القاتلة الطبيعية قد يكون بمثابة خلية قامعة و هي أيضا خلايا تنظيمية تعمل في التفاعلات المتبادلة مع الخلايا الجذعية، الضامة، وخلايا T والخلايا البطانية.

## INTRODUCTION

The mammalian placenta serves as an immunologic barrier between the maternal and foetal circulations, preventing the potentially destructive maternal immune response from damaging the semiallogeneic foetus, which is a mating product of non- histocompatible individuals, and not to be rejected.<sup>[1,2]</sup> The mechanisms by which the foetal trophoblasts circumvent the maternal immune response in the pregnant uterus have been investigated,<sup>[3,4]</sup> although there is still no generally accepted explanation for this phenomenon, it has been suggested that maternal immune cells are actively recruited to the decidua to exert an immunoregulatory effect on growth, and function of the placenta.<sup>[5,6]</sup> The first barrier between the invasive trophoblasts and the circulating cells of the maternal immune system is the maternal endothelium of local vessels, and specialized mechanisms may exist which act to regulate leukocyte extravasation into the deciduas,<sup>[6]</sup> and also because the placental structure is a site of the expression of trophoblastic antigens, therefore, the foetal/uterine relationship may change dramatically during pregnancy and that may further play a critical role in determining the nature of local immune responses at different stages of gestation.<sup>[7]</sup> Placental trophoblastic cells are in close contact with the maternal tissues forming the so called foeto- maternal interface,<sup>[8]</sup> and are essential for modification of the maternal uterine environment into a hospitable site for embryonic and foetus development.<sup>[9]</sup> Large mononuclear or binuclear cells with abundant prominent granules were described long time ago by morphologists studying implantation sites of pregnant rodents. These striking cells accumulated by midgestation in large numbers in a structure unique to rodent pregnancy that develops in the

mesometrial region of the uterine musculature and was given the name of Metrial Gland. Thus, the cells were originally termed Granulated Metrial Gland (GMG) cells.<sup>[10]</sup> Evidence provided over the last years, has suggested that these cells are bone marrow-derived lymphoid cells which differentiate in situ in the mouse pregnant uterus into natural killer (NK) cell lineage and they are referred to as granulated uterine NK (uNK) cells.<sup>[11]</sup> In the human uterus, the defensive cells present at the foeto- maternal interface are macrophages and uNK cells which commonly called Decidual Granular Leukocytes (DGLs) or Endometrial Granulated Lymphocytes (eGLs, possibly natural effector cells which have an unusual phenotype and they seem to accompany the invading trophoblast.<sup>[12]</sup> A recent study suggested that the activity of GMG cells and peripheral NK cells might be influenced by the rat's gene, which is involved in reproductive performance and successful pregnancy in rats.<sup>[13]</sup> The aim of this study is to demonstrate any further evidence which may lead to the suggestion that the granulated metrial gland (GMG) cells are specialized immune cells which are involved in inhibition of the rejection reaction of the mother to her foetus as an allograft in pregnant mice uterus and it's relation to the decidual granular leukocytes (DGLs) in human pregnant mothers during early pregnancy.

## MATERIALS AND METHODS

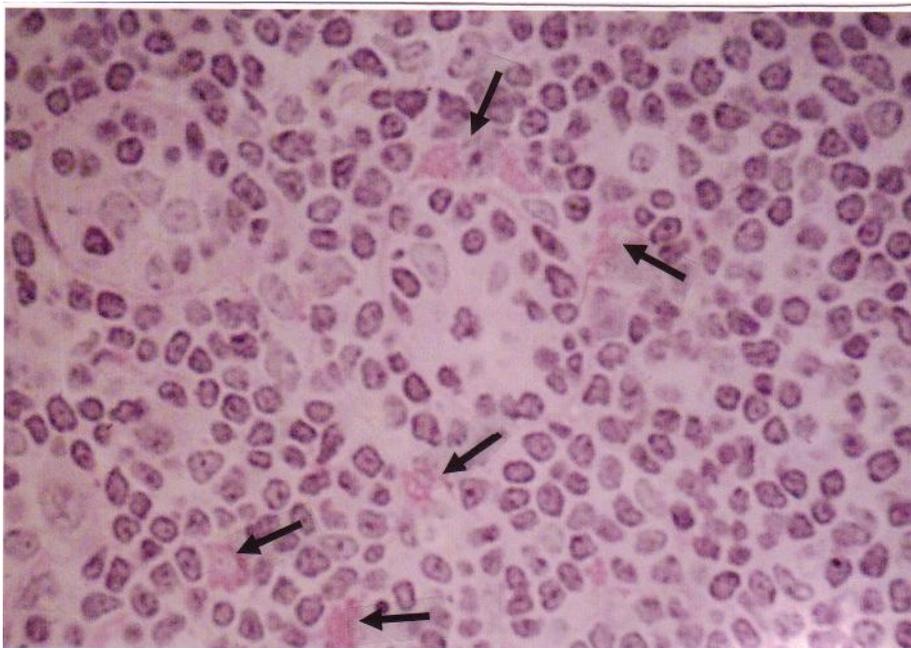
A total of thirty nulliparous pregnant female albino mice of BALB/c strain aged 8 weeks were mated overnight with a male of the same strain. Females were examined every morning for the presence of vaginal plug, the morning on which vaginal plug was found was taken as day 0 of pregnancy. Animals (at least five), were anesthetized then killed by cervical dislocation

on each of days (6, 10, 12, 14 and 16) of pregnancy at 9:00 am. Caesarian sections were done by a longitudinal incision to expose abdominal contents. The uterine horns were exposed under aseptic conditions; a longitudinal incision was made along the amniotic sac, exposing the foetuses that were extracted. The placentae with implantation sites from the uteri were carefully shelled out and the remaining decidual tissue was peeled out from the surface of the metrial gland which was then separated from the uterine muscle. This study also included human aborted placental tissue samples, obtained by curettage process of different normal pregnant mothers who had abortion and termination of their pregnancy. All specimens were fixed in a mixture of 10% formalin as a fixative solution. Tissue sections were cut of about (3-5)  $\sim m$ , stained by using

(basic and acidic) stains hematoxylen and eosin (H&E), and periodic Acid Schiff (PAS) technique by using SCHIFF'S REAGENT. All preparations were examined with the light microscope, each slide was examined carefully for the presence of the GMG & DGLs cells and selected images were taken by using of special light microscope provided by photographic camera and the digital camera also used to clarify the fine details.

## RESULTS

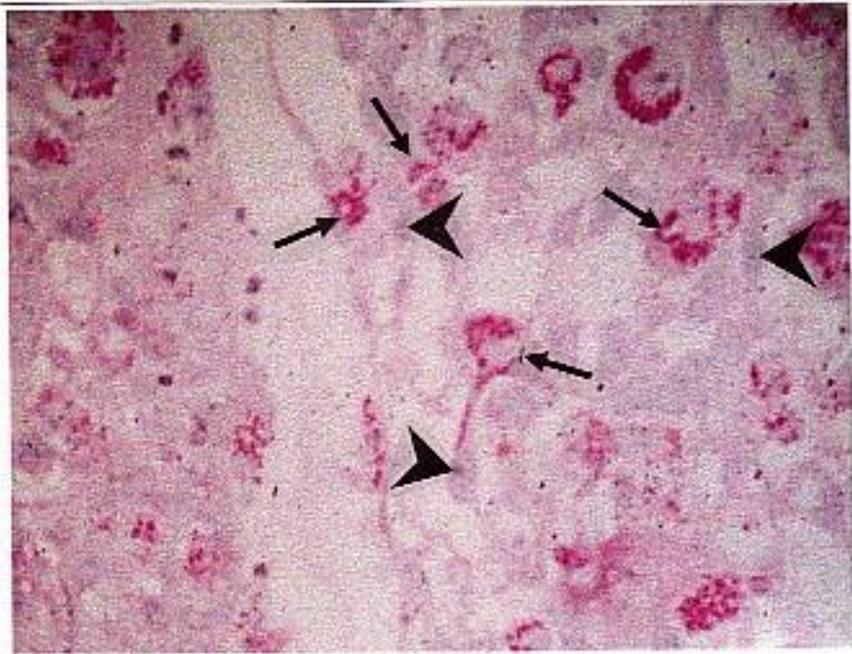
The main results obtained from this study, *regarding mice implantation sites*, were: At **day 6 of pregnancy**, there were few granulated metrial gland (GMG) cells found in the endometrium randomly distributed as shown in (Fig.1).



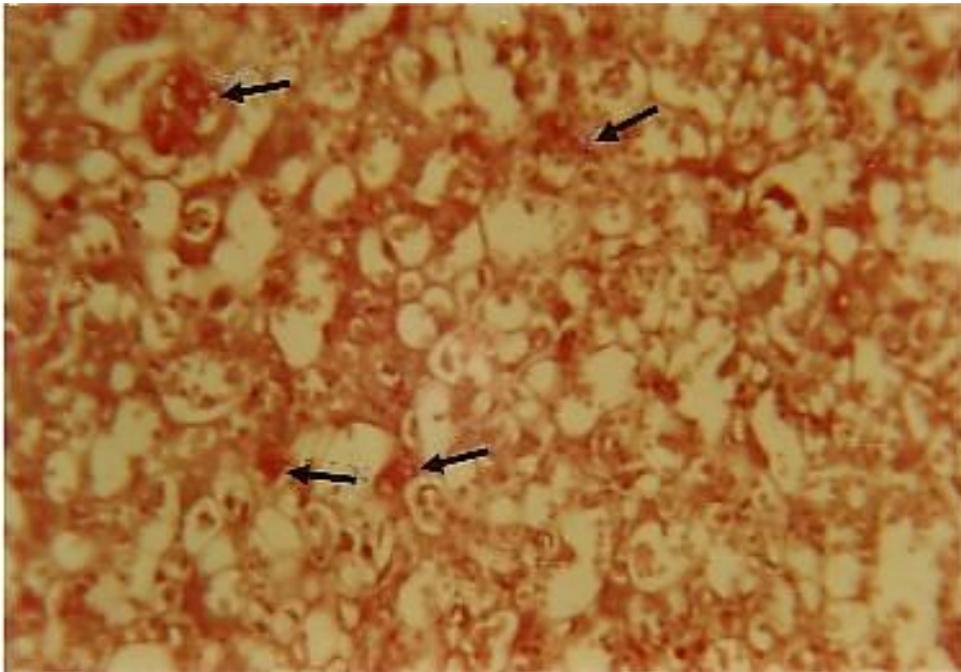
**Fig 1. Section of the mouse uterus at day 6 of gestation shows few granulated metrial gland (GMG) cells randomly distributed throughout the endometrium (arrows). X 742.5**

At **day 10 of pregnancy**, the GMG cells were observed populated at the region of the mesometrial triangle of the uterine musculature which is the region of the characteristic metrial gland. Some GMG cells were found in the maternal blood vessels of metrial gland as in(

Fig. 2). Some of them were also seen in the developing decidua basalis of the uterus closely related to the fibroblast-like stromal cells (Fig-3). While, at **day 12 of pregnancy**, the GMG cells were seen largely distributed in the area of metrial gland.



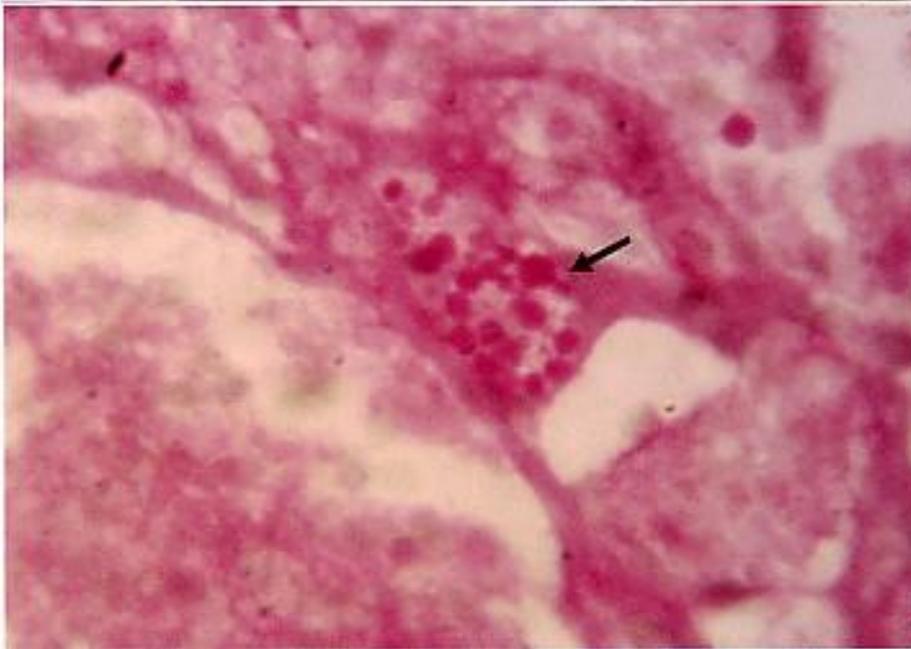
**Fig 2. Granulated metrial gland (GMG) cells in the region of metrial gland at day 10 of pregnancy (arrows ). X742 . 5**



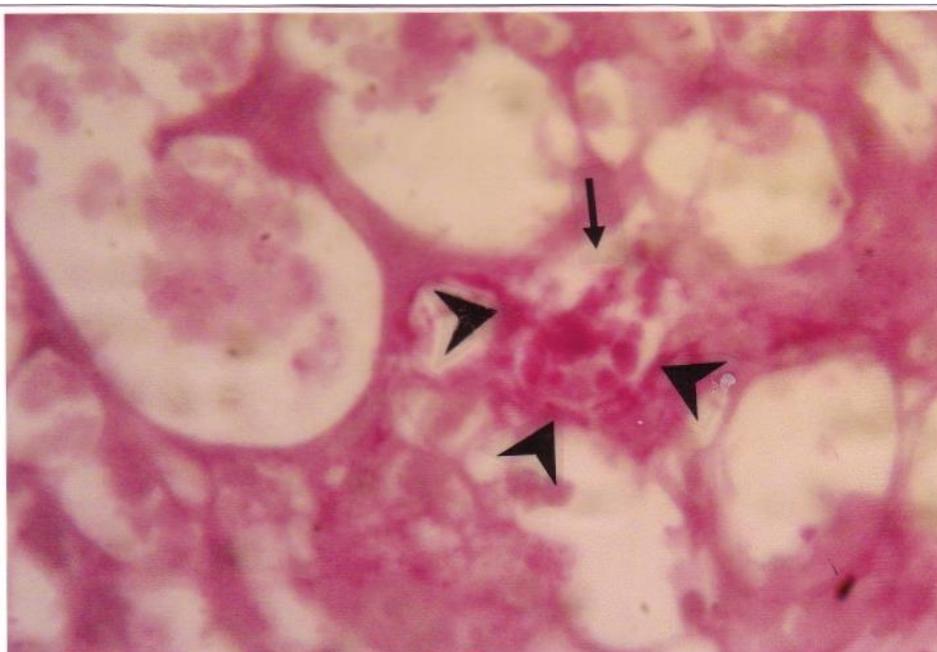
**Fig 3. Section of the mouse uterus at day 10 of gestation shows GMG cells (arrows) closely related to the fibroblast – like stromal cells (arrow heads). X 1856.5**

At **day 14 of pregnancy**, at this stage of pregnancy a considerable number of GMG cells appeared to lie free in the blood vessels of labyrinthine placenta (Fig-4). Other granulated

cells showed an intimate contact to the trophoblast layer I, both the GMG cells and the trophoblasts cells appeared in a healthy intact state (Fig-5).



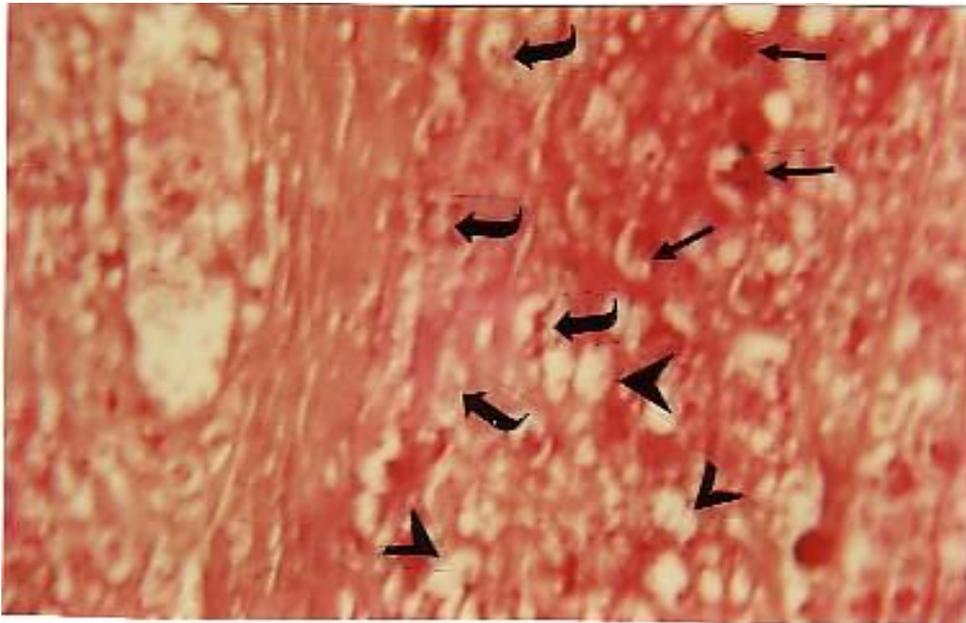
**Fig 4. The labyrinthine placenta at day 14 of gestation with GMG cells (arrows) freely located within the labyrinthine blood vessels**



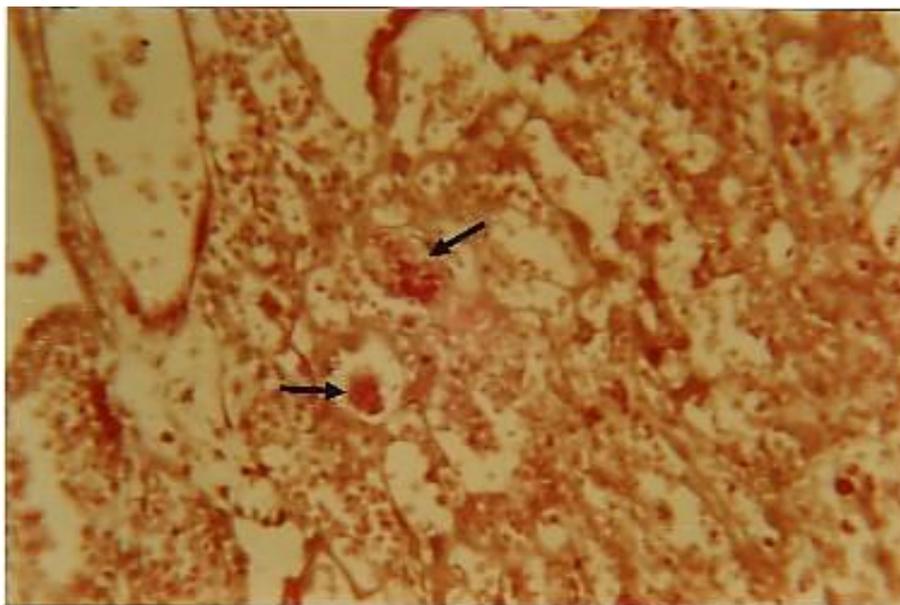
**Fig 5. Section of the labyrinthine placenta at day 14 of gestation shows GMG cells (arrows) in intimate contact with trophoblastic layer I (arrow heads) . X 1856 .25**

At **day 16 of pregnancy** a reduction in the populations of GMG cells was observed in the area of metrial gland, mostly appeared unhealthy and disrupted and this was indicated by the appearance of cytoplasmic vacuoles and some of these cells were associated with disorganisation of their characteristic

cytoplasmic glycoprotein granules with variation in their electron density. The nucleus appeared pyknotic (Fig.6). Few GMG cells were observed in the maternal blood spaces of the labyrinthine placenta, but generally they appeared unhealthy.(Fig.7).



**Fig 6.** The metrial gland at day 16 of gestation with reduced populations of GMG cells (arrows) and empty spaces (arrow heads), other GMG cells look unhealthy with faintly stained cytoplasmic granules and vacuolated cytoplasm (bend arrow. X 742. 5

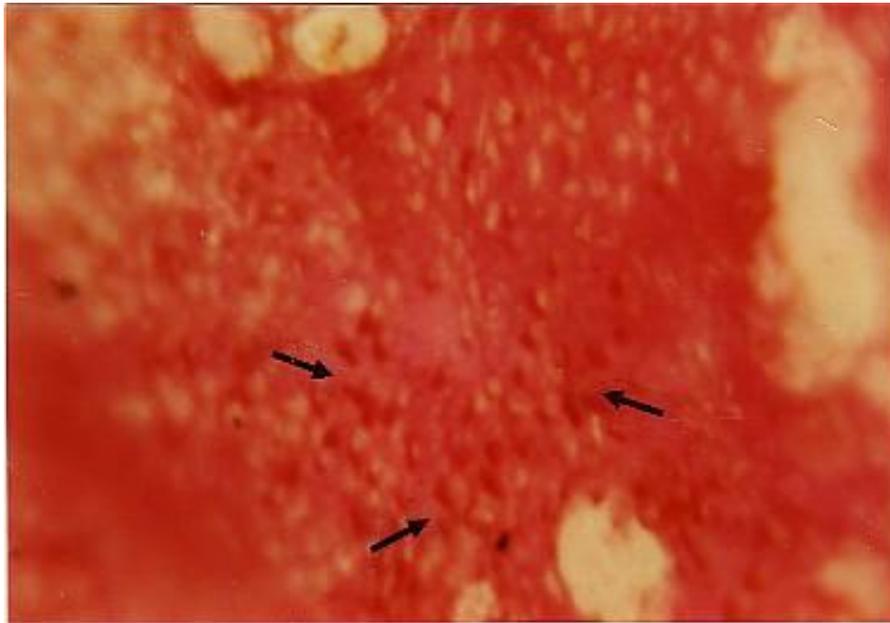


**Fig 7.** The labyrinthine placenta at day 16 of gestation with unhealthy vacuolated GMG cells in the maternal blood spaces (arrows) P.A.S. X 742. 5

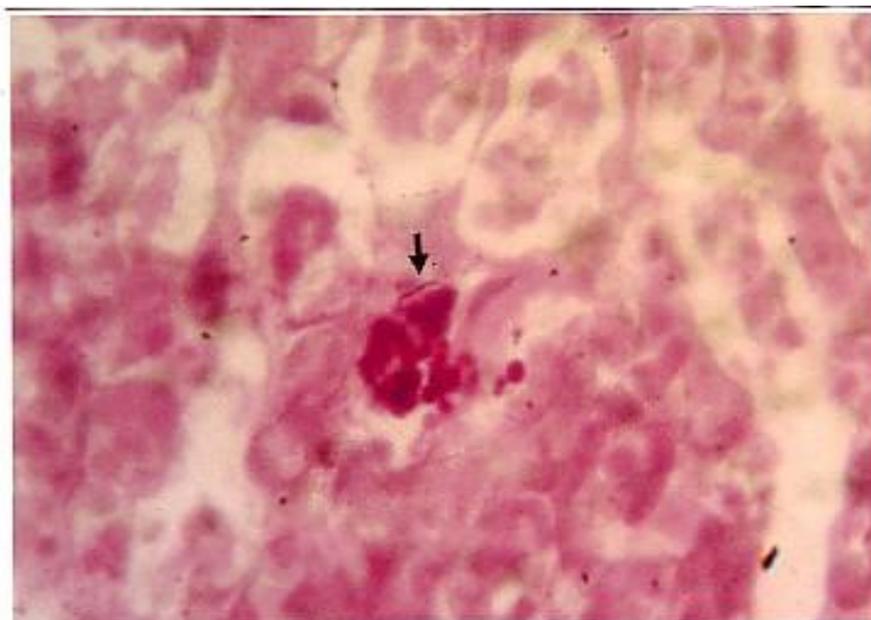
***Regarding human aborted placental tissue samples,***

In **2 months aged placenta**, large populations of decidual granular leukocytes (DGLs) or endometrial granulated lymphocytes (eGLs), which looked similar in structure, size and characteristic features to the granulated metrial gland (GMG ) cells as described in mice by

several investigators, were found in human placenta. These cells were seen in large populations at this stage of human placenta, they looked healthy with their large size and their large abundant characteristic P.A.S. positive cytoplasmic granules. Some of DGLs cells were observed within the maternal blood vessels as shown in (Fig.8 & 9).



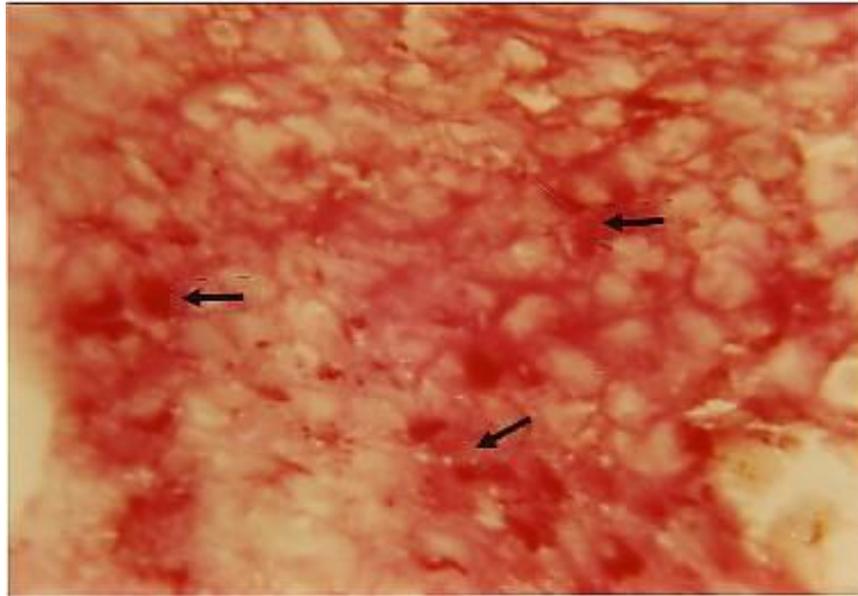
**Fig 8. Aborted human placenta at 2 months pregnancy with large populations of decidual granular leukocytes (DGLs) (arrows). X 742. 5**



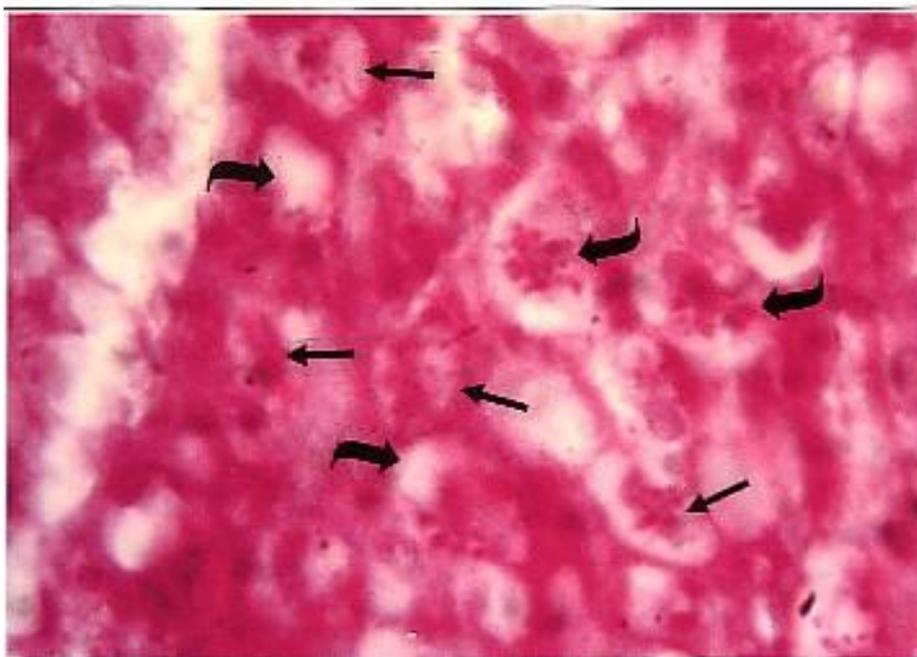
**Fig 9. Two months aged aborted human placenta with DGLs cells within the maternal blood vessels (arrows) with their P.A.S. positive cytoplasmic granules. X 1856. 25**

In **3 and 5 months aged placenta**, the placenta appeared with reduced populations and distribution of DGLs (a behavior similar to that of the mouse GMG cells at day 16 of gestation), they looked unhealthy (Fig.10). Some of these

cells observed with vacuolation and disorganization of the cytoplasm, others showed little cytoplasmic granules with variable degree of reaction of their cytoplasmic granules to P.A.S. stain as shown in (Fig. 11).



**Fig 10. Aborted human placenta at 3 months pregnancy with reduced populations and unhealthy DGLs (arrows). X742.5**



**Fig 11. Section of aborted human placenta at 5 months pregnancy shows reduced populations of DGLs with reduction in the number and reaction of their cytoplasmic granules to P.A.S. stain (arrows) and vacuolation of the cytoplasm (bend arrows). X 1856. 25.**

## DISCUSSION

From immunological point of view, pregnancy is an innate immunity event and one of its components are the GMG cells with their unique properties, among the cells of the immune system and are particularly suited for specific effectors mechanisms combined with immunoregulatory functions. The GMG cells in rodents pregnancy are the temporary inhabitants of the decidual tissue and labyrinthine placenta differentiate locally in uterus from a bone-marrow derived precursor cells, they have the potency to protect the fetus from being rejected, thus modulate the maternal immune system towards tolerance of her fetus. Evidence might be obtained by finding of differences in the distribution of GMG cells in the pregnant uterus of mice at different stages of pregnancy. For instance, at day 6 of pregnancy, there were very few populations of GMG cells randomly distributed throughout the endometrium, then around day 10 of gestation, a rapid increase in the distribution of the granulated cells was observed in the mesometrial triangle which is the site of metrial gland and in the deciduas basalis, the presumed source of differentiation of granulated cells, this might indicates that these cells are potentially available to start migration to the labyrinth and because the labyrinthine placenta has not yet established or formed at this stage.<sup>[14]</sup> While at day 12 of gestation, a striking influx of GMG cells was found in the area of metrial gland, the GMG cells reach their maximal accumulation at day 14 of pregnancy in the labyrinthine placenta reaching their peak with the peak of placental development because the labyrinth is fully formed at this time.<sup>[15]</sup> The significant increase in the populations of granulated cells at the peak of labyrinthine development and the cells interaction which has been detected between granulated cells and the labyrinthine trophoblast suggests that the labyrinthine placenta is an important site in relation to the function of granulated metrial gland cells. At day 16 of pregnancy, when placental growth has ceased and the process of placentation is completed at this stage,<sup>[16]</sup> there was a great reduction in the population of GMG cells in the metrial gland and decidua basalis and also disappearance of some GMG cells from implantation sites was also observed. The few GMG cells found in the

maternal blood spaces of labyrinthine placenta undergone several morphological changes which are indicated by degeneration and cell death of GMG cell. The loss of the granulated cells may be due to their degeneration in situ which has been reported by previous workers and it has been suggested that this may be of functional significance.<sup>[17,18]</sup> A recent study suggested that the activity of rat GMG cells and peripheral natural killer (NK) cells might be influenced by the rat's gene, which is involved in reproductive performance.<sup>[13]</sup> The class I major histocompatibility complex (MHC) receptors expressed by NK cells play an important role in regulating their function.<sup>[19]</sup> A supporting evidence demonstrates that the cellular composition, morphology, and immunohistochemical staining profile of normal metrial glands are similar to reported granular cell neoplasms in rats and mice.<sup>[20]</sup> Human decidua is a leukocyte-rich tissue, macrophages, NK cells, T-cells and other decidual granular leukocytes (DGLs) or uNK cells are the most abundant leukocyte populations in decidua of the first trimester. DGLs account for up to 70% of endometrial leukocytes<sup>[21]</sup>, 10-15% of all cells are leukocytes.<sup>[22]</sup> The results also revealed an increase in the populations and distribution of DGLs in human aborted placental tissue, they were seen in placental tissue at 2 months more than the 3&5 months. Recently, a supporting evidence has reported that the increase in number of uNK cells at the implantation site strongly suggests that uNK cells are involved in the maintenance of normal pregnancy.<sup>[23]</sup> Human decidua from early pregnancy between days 18 and 38 (postcoitum) of pregnancy contains numerous DGLs and their aggregates are active against the semi – allogeneic embryo, Natural killer (NK) cells are vital effectors cells of innate immunity because of their rapid cytotoxic and cytokine-producing responses to cell stress or infection.<sup>[24]</sup> Since these cells are possibly natural suppressor cells and probably protect the materno-foetal unit from other effects causing disorders to placental development. Immunosuppressive activity has been demonstrated in the first trimester of human decidua and it's possible to play a role in preventing maternal immunologic attack on the allogeneic embryo. Thereby, preventing

spontaneous abortion, hence DGL may act as a suppressor cell.<sup>[6]</sup> Recent research highlights the fact that NK cells are also regulatory cells engaged in reciprocal interactions with dendritic cells, macrophages, T cells and endothelial cells.<sup>[25]</sup> It is possible that DGL or their soluble products play a role in the outcome of human pregnancy because their number appeared to be increased in spontaneous abortion.<sup>[26]</sup> The immunological mechanisms that contribute to the maintenance of normal human pregnancy and determine the failure of a pathological pregnancy remain poorly understood. The immunosuppressive activity of DGL in spontaneous abortion needs more investigations to clarify whether this role is critical for maintenance of pregnancy.

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