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ULTRA-STRUCTURAL CELLULAR ALTERATIONS CORRELATED WITH CHRONIC ANEMIA IN RAT PLACENTA

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Abstract:

Objective: *Anemia due to deficiency of iron is the cause of the adverse outcome of pregnancy. There are some research works on the impacts of anemia on the degenerative changes in the trophoblastic cells which are very important in the function of placenta. This present data is very vital for the elaboration of the function and complications of placenta. This research work aimed to interrogate the degenerative alterations in the trophoblastic cells linked with the anemia due to iron deficiency in the rat placenta.*

Methodology: *In this research, we randomly divided the 49 Sprague-Dawley female rats in the group of experiments and group of healthy control. The 1st group was extracted anemic by takeout 1.5 milliliters blood per bleed on 5 alternate occasions, and we collected the placentas on 17th, 19th and 21st gestation day. We fixed the 5 cubic mm segments in solution with of 10% buffered formaldehyde; ethanol-dehydrated and set in paraffin wax for the light microscopy. We cut the thick sections of 5 microns, deparaffinized and performed their staining with the Eosin and Hematoxylin. For the inculcation of the TEM (Transmission Electron Microscopy), we fixed the one mm3 sections in 2.5% glutaraldehyde buffered with phosphate, post-fixed in 2% osmium tetroxide, dehydrated carried out in ethanol, cleared it in the propylene and set in the epon resin. We stained the ultrathin sections with the help of uranyl acetate and then examined the lead citrate with the utilization of the JEOL electron-microscope.*

Results: *The giant trophoblastic cells, cyto-trophoblast and syncytio-trophoblast of the rat placentas present with anemia displayed nuclear and cytoplasmic vacuolation with the damage to the cell margins. Additionally, we found the microvilli atrophy on the surface of the cell as well as Nuclear Chromatolysis, ND (Nucleolar Degeneration) and presence of the dark bulks.*

Conclusion: *Chronic iron deficiency anemia is the most important reason of the degeneration of the trophoblastic cells. This issue has the ability to weaken the operational integrity of the affected cells and they become the part of the process which can cause the adverse outcome of pregnancy.*

KEYWORDS: *Anemia, Ethanol, Hematoxylin, Transmission Electron Microscopy, Uranyl Acetate, Placenta.*

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INTRODUCTION:

Syncytio-trophoblast and cyto-trophoblasts cells in zone of labyrinthine and spongio-trophoblast which are giant trophoblastic cells present in the junctional zone [1-4]. The latter played the main role in the constitution of the inter-hemal membrane or the barrier of placenta. Anemia is an important reason behind the adverse outcome of fetal [5-8]. This adverse outcome has been credited to the degenerative alteration occurring in placenta as decrease in the size, reduced vascularity, syncytial knots, fibrinoid necrosis and infarcts in placenta [2, 9, 10]. For the functioning of placenta, the role of the trophoblastic cells cannot be under-estimated and these cells are very sensitive to any anomaly in placenta [11–13]. The awareness of the impacts of various abnormalities is very important in the mitigation of the adverse outcome of fetal. Research works on the ultra-structural alterations due to anemia in the trophoblastic cells of placenta are very rare. So, this research work carried out to investigate the impacts of anemia on the ultra-structural alterations in the trophoblastic cells of placenta.

MATERIAL AND METHODS:

This research work carried out on 49 Sprague-Dawley female rats. We divided the samples into two groups of controls and experiment. We formulate the directives to secure the beneficial environment and care for the samples. Some of the basic principles for the care of these animals are; optimum conditions for living, reasoning no stress or pain, utilizing less invasive procedures, medical care, performing no tests which are not necessary, decreasing the amount of the animals under testing to minimum. We handled the animals with care with no frightening to the animals. We induced the chronic anemia with withdraw of 1.5 milliliters blood on 5 alternate days, by the use of the retro bulbar plexus in accordance with the guidelines of Markova presented in 2009 [14].

We diagnosed the anemia when there was a drop in the level of hemoglobin below 12 g/dl. The average level of hemoglobin for rats of control group was 13.1 g/dl with a range from 12.87 g/dl on day seventeen to 13.4 g/dl on day 21. In anemia, the level of hemoglobin was below 12 g/dl with a range from 10.98 g/dl on day seventeen to 11.44 on day 21. We found the statistical significant disparity on all the dates of gestation period. The rats with chronic anemia were present with average values of the concentration of hemoglobin that was much less as compared to the average values in the group of controls as presented in Table-1.

Table I: Effect Of Chronic Anemia On Hemoglobin Levels

Gestation day	Hb (g/dl)				p-value
	Control		Chronic		
	Mean	SD	Mean	SD	
Day 17	12.88	0.23	10.98	0.69	0.039*
Day 19	12.98	0.16	10.67	0.5	0.001*
Day 21	13.4	0.5	11.44	0.58	0.013*

*p<0.05

We anesthetized the samples with the use of ether inhalation, we used the midline incision to open the abdomen, exposed the uterus and we extracted the placenta on days 17, 19 and 21 (Table-2). We cut all the placenta into 2 equal parts. Light microscopy was in use for the process of the half and other half underwent TEM (Transmission Electron Microscopy) for processing. We performed both of the procedures with international guidelines.

Table II: Distribution On Rats On Different Gestational Days

Day	No of Rats	
	Cont. Group	Exp Group
17	6	5
19	5	8
21	13	12
Total	24	25

We carried out the dehydration in rising ethanol grades, embedded in the epon resin and cleared in the propylene oxide. We cut down the semi thin sections at about 500 to 1000 nm thickness and then we carried out the staining with the use of toluidine blue. The staining of the ultrathin sections carried out with the uranyl acetate with counter staining using lead citrate. We carried out the examination using JEOL TEM at different magnifications with the use of the instructions of Hunter [15] and Bazile and Russel [16]. We took the images with camera with high resolution.

RESULTS:

There were visible margins, nuclei and cytoplasm in the trophoblastic cells present in placenta of animals of group of controls. There were numerous, definite and long microvilli on the cyto-trophoblasts. There were degenerative features on the placenta of the animals present in the placenta of the animals of anemia group. Nucleus of TC present in the inter-hemal membrane displayed the Chromatolysis features such that there was disappearing of the chromatin material from nucleus. The margins of the cells were not clear. This was in opposition to the animals of control group in which the cells were present with obvious margins, visible chromatin and nucleus (Figure-1a and Figure 1b). TC in inter-hemal membrane showed many dark bodies, disorganization, vacuolation and degenerative impact (Figure-1c and Figure-1d).

The syncytio-trophoblasts displayed the degeneration of the nucleolar and chromatin as well as cytoplasm and nuclear vacuolation (Figure-2a and Figure 2b). There was development of the nuclear vacuolation and dark bodies in the cyto-trophoblasts. Surfaces facing the MBS bore microvilli which decreased in amount and cells were present with various orientations cyto-trophoblast surface (Figure-2c and Figure 2d).

In cases of anemia, there was loss of the margins of cells, cytoplasm and nucleus with the disappearing of the nucleolus (Figure-3a and b).

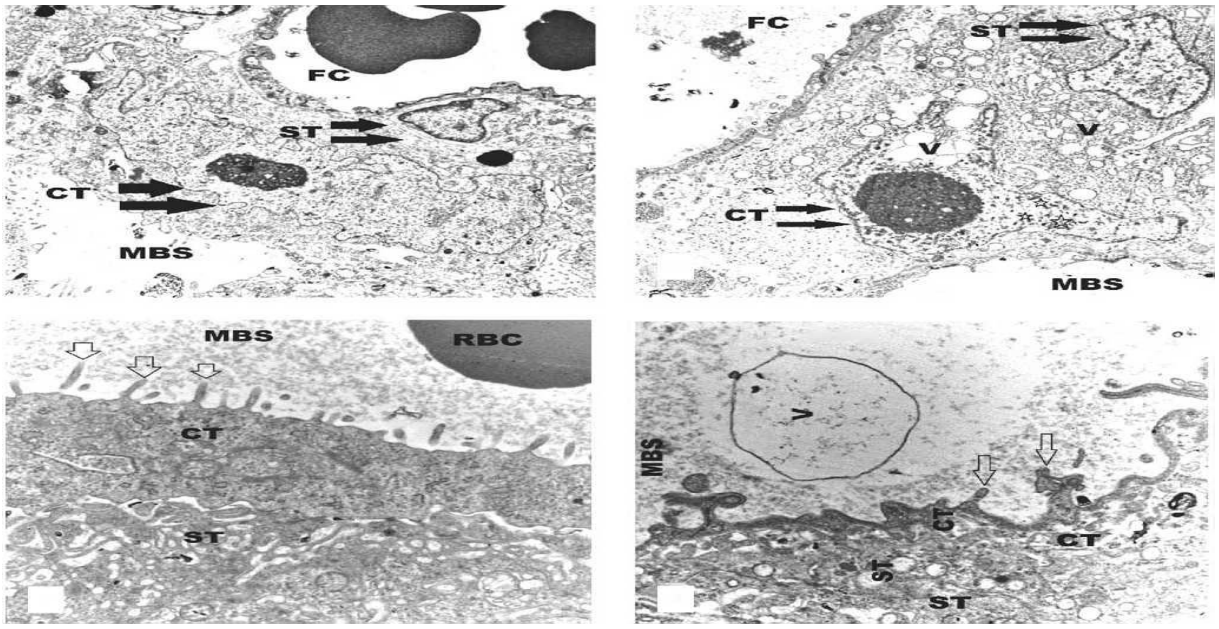

Figure 1

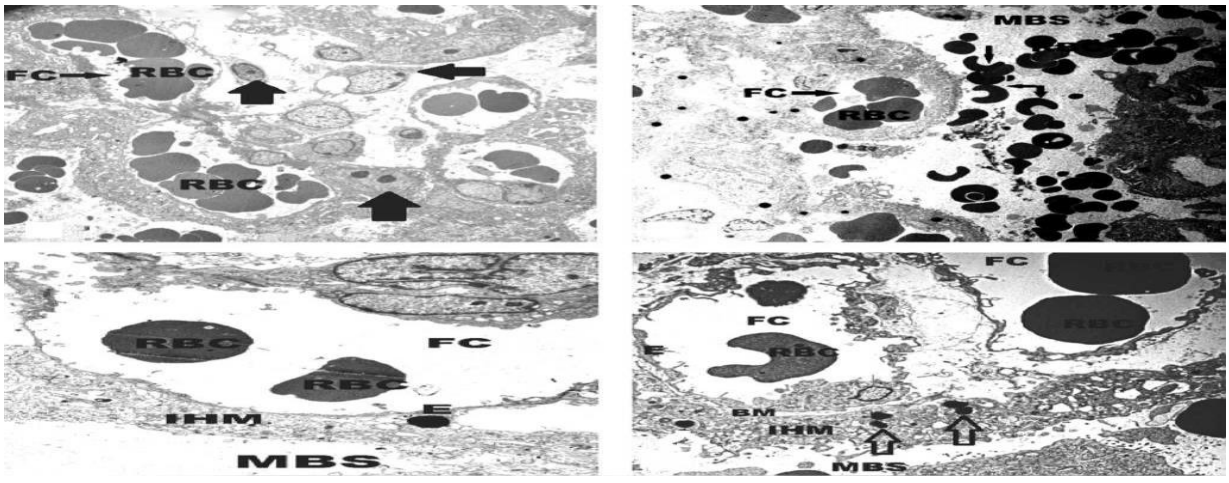

Figure 2

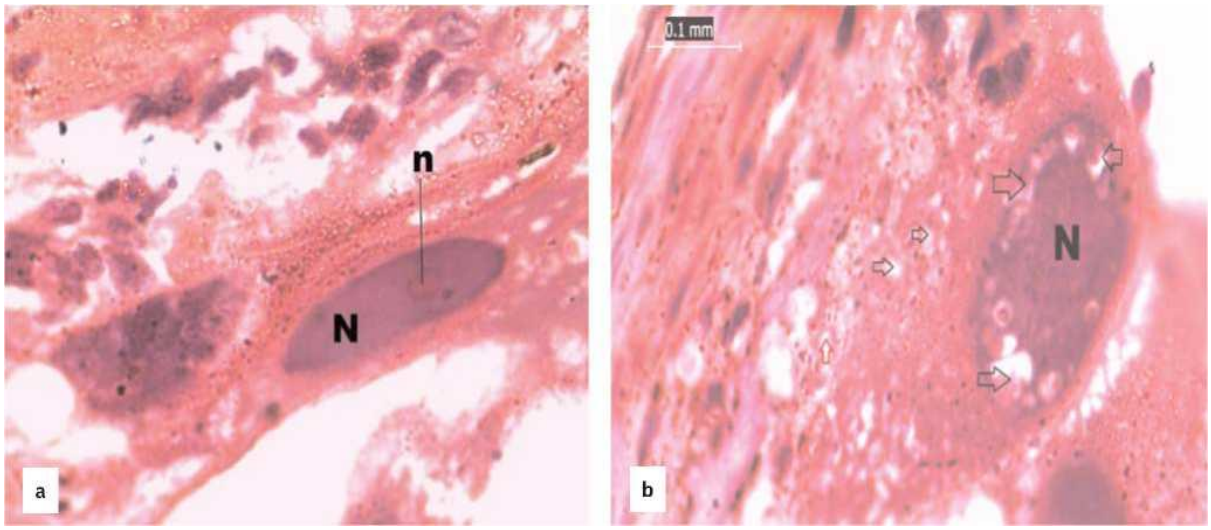

Figure 3

There was much less amount of the giant trophoblastic cells in the group of animals with anemia in comparison with the group of healthy controls (Figure-4a and Figure-4b).

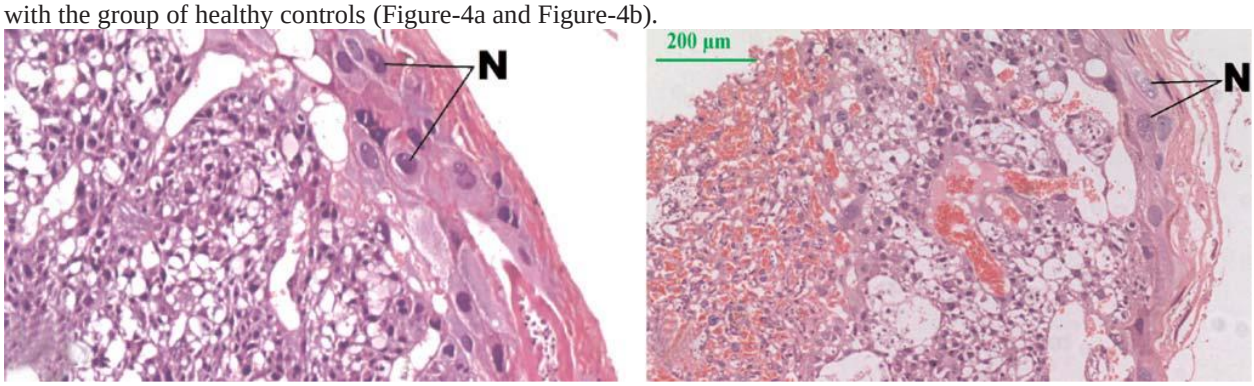

Figure 4

DISCUSSION:

Cyto-trophoblasts, syncytio-trophoblasts and giant trophoblastic cells were the cells which were largely affected due to anemia. Same alterations of degeneration in these particular cells were present in the reports about hypoxia [17, 18], preeclampsia [19] and hypertensive complication in the period of pregnancy [20]. These alterations are much concordant with the research reports that these very cells are much sensitive to the features which are responsible for the injury of placenta [13, 21, 22]. Majority among them, particularly those having relation to the hypoxia occur because of the apoptosis of placenta which is the outcome of the oxidative stress [23]. The activity having involvement of the metabolites transport from and or to the fetus has the participation of syncytio-trophoblast as well as these are also important for the hormones production factors of growth and other factors responsible for the regulation of the maternal metabolism and development of the placenta [11, 24].

Reduced density of the microvillus as discovered in this research work is also present in other abnormalities as IUGR placentae [25] and pre-eclampsia [17]. Cyto-trophoblasts in rats continuously differentiate into ST during the development and formation of the villous and they are much vital in the invasion of remodeling of blood vessels in initial implantation stages [23]. The secretion of the steroid hormones and cytokines related to prolactin carried out by trophoblastic giant cells which play an important part in the placentation. In this very research work, these cells displayed the nuclear and marked cytoplasmic vacuolation. Same alterations of degeneration are reported in the complications of DM (Diabetes Mellitus) [22] and chemical toxicity of placenta and restriction of the gestational protein [25]. All these changes in the morphology can have adverse impacts on the fetal outcome.

CONCLUSION:

Chronic iron deficiency anemia is the most important reason for the degeneration of the trophoblastic cells. This issue can have affect the

functional integrity of these affected cells and these cells make the mechanism for prompt of the adverse outcome of pregnancy.

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