

## Histological changes of placentas associated with intra-uterine growth restriction of fetuses: a case control study

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### ABSTRACT

Placenta is the maternal-fetal contact zone. The placentas of "idiopathic" intra-uterine growth restriction (IUGR) babies may hold the key to the etiology of growth restriction. The present study primarily aimed at evaluating the structural peculiarities of IUGR placenta. The study was conducted on 35 IUGR and 25 control placentas. Placental tissues were processed for routine histological studies, to determine numbers of syncytial knots in villi and X-cells in the basal plate. Light microscopy suggested that syncytiotrophoblastic lining was more degenerated and number of syncytial knots increased in IUGR placentas than that of the control placentas. X cells were present in both the cases, though more in IUGR. Intravillous and perivillous fibrin depositions were markedly increased in IUGR; also there were more hypovascular / avascular villi and large areas of infarction. Cumulative effects of several placental injuries, e.g. poor perfusion, presence of increased number of X-cells, increased fibrin deposition; etc for a sufficient time were likely cause of IUGR.

**Keywords:** placental histology, IUGR, syncytial knots, X cells

### INTRODUCTION

Placenta is the maternal-fetal contact zone, provided by fetal membranes and endometrium. Intrauterine existence of fetus is dependant on the placenta. It is elaborated by both maternal and fetal tissues, and serves as an instrument of transfer of essential elements, i.e., nutrients and oxygen from mother to embryo, and the waste products of metabolism from embryo to the mother.

The one and most important cause of neonatal loss is the *low birth weight* (birth weight 2500 gm or less), irrespective of gestational age. Intrauterine growth restriction (IUGR) or Small-for-Date (SFD) babies are the low birth weight babies having impaired growth rate which is disproportionately low for that gestational age (<10<sup>th</sup> percentile). "Idiopathic" intrauterine growth restricted babies are those who do not have any obvious fetal or maternal cause for growth restriction.

The placentas of the "idiopathic" IUGR babies may hold the key to the etiology of the growth retardation, though the contributions of placental changes remain controversial. Contradictory histological and morphological findings were recorded while comparing the placentas from pregnancies delivering intrauterine growth restricted babies with those delivering normal-weight babies.<sup>1</sup> Extensive perivillous fibrin deposition, maternal floor infarction, widespread thrombosis of fetal arteries were associated with IUGR but such lesions were

found on only a small minority of placentas from growth retarded fetuses.<sup>2</sup> Others found increased numbers of syncytial knots and proliferation of X-cells in placentas of IUGR fetuses.<sup>3,4</sup>

The present study primarily aimed at evaluating the structural peculiarities of placenta and associates those with IUGR babies.

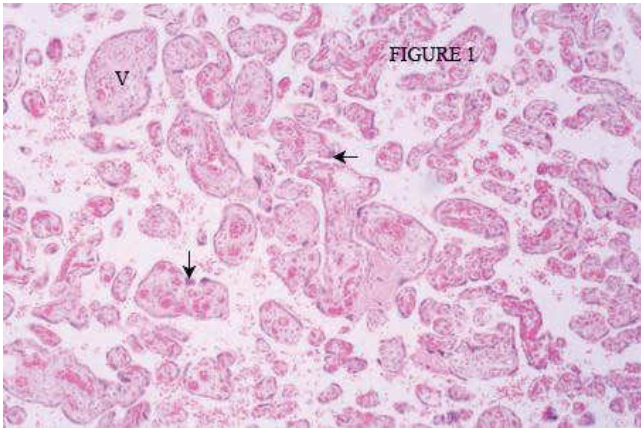
### MATERIALS AND METHODS

The study was conducted on a total of 60 placentas from full-term deliveries (37-42 weeks). Of these, 35 placentas were associated with intrauterine growth retarded fetuses (Birth weight < 2500 gm); and 25 placentas were from normal-weight babies taken as control group (Birth weight > 2500 gm). All the babies were delivered either normally or by Caesarian section and did not have any prenatal, intranatal and postnatal complications. Gestational ages were established from Last Menstrual Periods, and sometimes from USG reports. From clinical data sheets, the mothers' obstetric and medical histories were taken. Inclusion criteria for mothers were those who did not have any complications like pre-eclampsia, diabetes, renal diseases, infectious diseases, etc during pregnancy, or history of prolonged intake of any drug, or any other addictions. Fetal weights were noted from the Clinical sheets. Babies with congenital anomalies were not included in the study.

The study was performed after obtaining clearance from

**Table -1 (a):** Showing numbers of syncytial knots and x- cells in *iugr* placentas in unit area

number of syncytial knots (iugr placentas)				number of x-cells in basal plate (iugr placentas)			
Total no. counted	Maximum in an area of 0.02 25 mm <sup>2</sup>	Minimum in an area of 0.02 25 mm <sup>2</sup>	Average in an area of 0.02 25 mm <sup>2</sup>	Total no. counted	Maximum in an area of 0.02 25 mm <sup>2</sup>	Minimum in an area of 0.02 25 mm <sup>2</sup>	Average in an area of 0.02 25 mm <sup>2</sup>
108	9	4	6	288	28	8	16
144	10	7	8	306	27	8	17
126	8	5	7	288	26	10	16
126	9	6	7	198	15	6	11
144	8	4	8	198	20	7	11
126	10	4	7	252	28	6	14
162	13	8	9	360	25	14	20
90	6	4	5	270	25	8	15
144	12	3	8	306	28	7	17
126	8	4	7	252	26	5	14
126	7	4	7	360	38	10	20
90	6	4	5	288	30	5	16
108	8	5	6	342	28	14	19
126	8	3	7	198	18	10	11
144	11	6	8	270	25	9	15
108	7	4	6	360	28	10	20
126	9	4	7	288	26	8	16
108	10	5	6	342	30	12	19
108	8	4	6	324	35	19	18
126	8	5	7	306	28	7	17
162	13	7	9	198	18	7	11
144	10	3	8	288	27	9	16
126	9	5	7	342	26	8	19
144	8	4	8	252	22	15	14
108	11	6	6	306	19	14	17
90	7	4	5	288	23	13	16
162	8	4	9	306	28	9	17
126	7	3	7	288	24	8	16
90	6	4	5	270	25	8	15
144	12	3	8	306	28	7	17
126	8	4	7	252	26	5	14
126	7	4	7	360	38	10	20
90	6	4	5	288	30	5	16
108	8	5	6	342	28	14	19
126	8	3	7	198	18	10	11

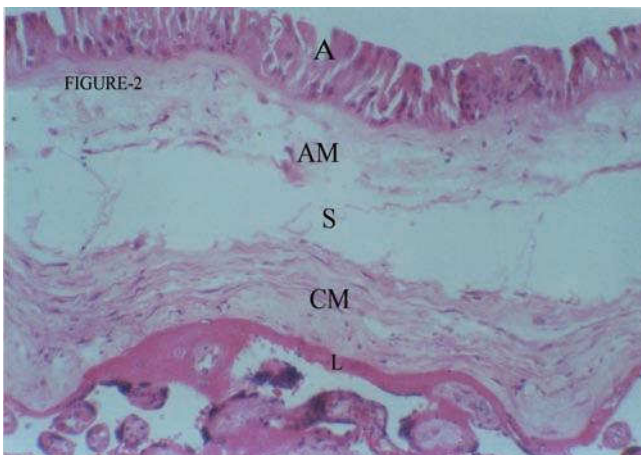


**Fig. 1.** Microphotograph of normal term placenta. Villi (v) are seen with syncytial knots (arrow) and capillaries are present within villi. H&E. X 100.

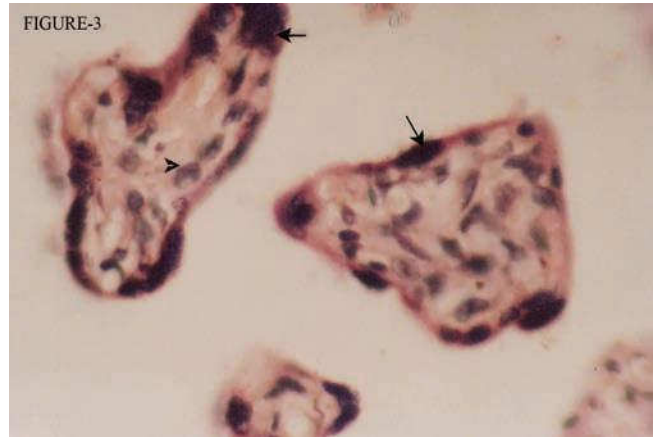
Institutional Ethical Committee. Before the placentas were collected, informed consents from the mothers were taken. The waste was disposed as per waste disposal norms.

Two pieces of placental tissues were cut, one from central and one from peripheral part and put in 10% formal saline for *fixation* for 3-4 days. Care was taken so that the sections were cut through the whole thickness of the placentas. The tissues were then processed routinely for histology. Seven micron sections were stained with Haematoxylin and Eosin stain, Masson's Trichrome and PAS stain. The slides were examined under light microscope in different magnifications. The basal plates, amnion and chorionic plates, chorionic villi and intervillous spaces of the placentas from both IUGR and control groups were studied and analyzed, and comparison between the two groups were made.

**Estimation of syncytial knots in villus and x- cells in basal plate:** For this purpose, optical apparatus comprising of Ermascope 202 along with an objective 20x was used. A square graticule drawn on an acetate



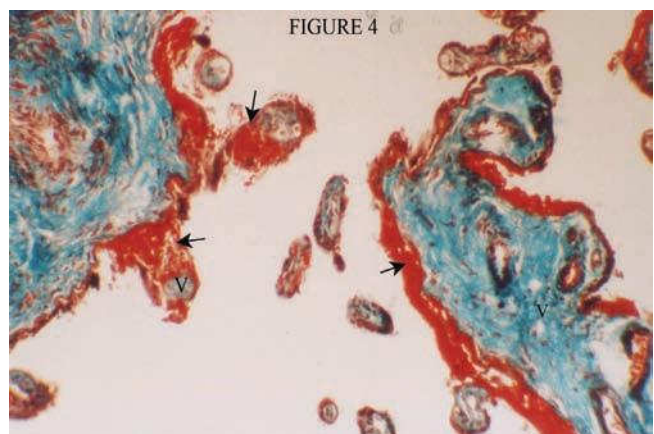
**Fig. 2.** Microphotograph showing chorionic plate with all the layers in normal term placenta:  
A – amnionic epithelium /AM – amnionic mesenchyme  
S – spongy layer CM – chorionic mesenchyme  
L – Langhan's layer of fibrinoid H&E. X 100



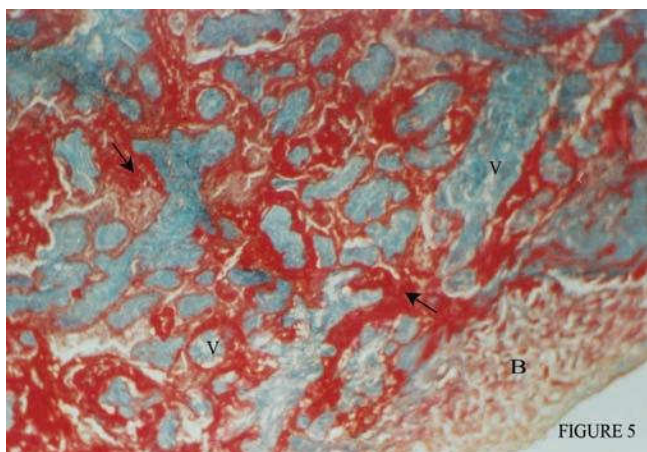
**Fig. 3.** Chorionic villi showing a faintly positive PAS stain in the villous core and basement membrane of the trophoblasts. Cytotrophoblast cells (arrow head) seen within the villous stroma. Syncytial lining is present at places. Syncytial knots (arrows) are plenty. H&E X 400.

sheet was placed on the ground glass projection surface of the Ermascope. The length of each side of the square was measured with the help of a linear stage micrometer, and it was found to be 0.15 mm. Hence, the area of the square graticule was  $0.15 \text{ mm} \times 0.15 \text{ mm} = 0.0225 \text{ mm}^2$ . This area was taken as unit area.

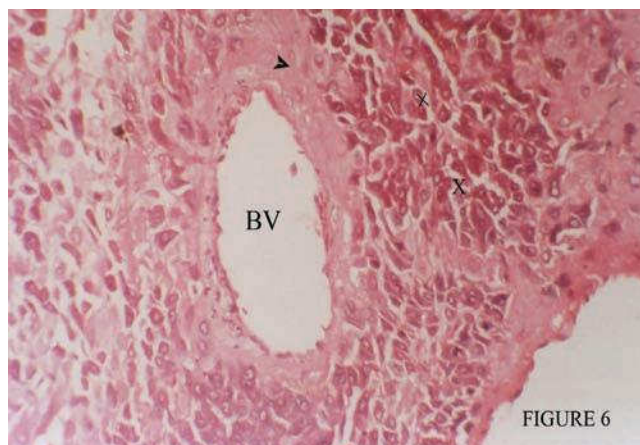
The numbers of syncytial knots falling in this unit area were counted. The numbers of X- cells present in the unit area of the basal plates were also counted. Data were collected by repeatedly changing the fields of placental sections. For counting syncytial knots in a unit area per villus, every third section was taken to avoid overlapping of the same syncytial knots. Total number of sections in each placenta was 10, altogether 30 fields were examined per placenta. Similarly, for counting number of X-cells in the basal plate, data were collected by examining 3 fields in basal plates per section, every third section was taken to avoid overlapping of the same X cells.



**Fig. 4.** Microphotograph showing that perivillous fibrin deposition (arrow), red in colour, has partially replaced trophoblastic lining of villi (v) in an IUGR placenta. Few villi (v) are seen to be entrapped in the fibrinoid. Masson's Trichrome. X 100



**Fig. 5.** Microphotograph of IUGR placenta showing massive perivillous fibrin deposit (arrow), red in colour, near the basal plate (B). The villi (V) are green in colour, avascular and sclerosed. Masson's Trichrome. X 100



**Fig. 6.** Microphotograph of the basal plate of an IUGR placenta showing plenty of large extra-villous trophoblastic cells (X-cells) (x) embedded in fibrinoid. Cross-section of an utero-placental blood vessel is seen (BV), its coats are replaced by fibrinoid (arrow-head). H&E X 400

## RESULTS

Placental histology was studied by light microscope. Variations of structures of villi, basal plates, chorionic plates and intervillous spaces at different regions of the same placenta, as well as of different placentas were noted.

In the control group of placentas (Fig. 1), syncytiotrophoblastic lining of the villi were thinned out and aggregated as syncytial knots. At places there were no trophoblastic cells in the lining, only basal lamina was seen. Intravillous fibrin depositions were present at places. Number of capillaries per villus was found to be 4 in an average. Perivillous fibrin depositions were noted in the marginal and subchorionic areas. Masson's Trichrome stain demonstrated presence of loose connective tissue, fibroblasts, few Hofbauer cells and fetal vessels.

Variable degree of villous crowding, incompleteness of the syncytiotrophoblastic lining, and congestion of villi were noted in some small areas.

Basal plates did not have typical layering. The surface of the basal plates facing the intervillous space was lined by syncytiotrophoblasts in patches, and was partly replaced by Rohr's fibrinoid. There were X-cells, and decidual cells in Nitabuch's fibrinoid. The X-cells were densely basophilic with granular cytoplasm and had large nucleus. Few fibroblasts were seen and also few mononuclear leucocytes (mostly lymphocytes). The *uteroplacental blood vessels* were noted to be lined with endothelial cells.

*Chorionic plates* had typical multi-layered structure, consisting of amniotic epithelium, compact layer, spongy layer, followed by compact layer of chorionic mesoderm (Fig. 2). Chorionic plates were lined by Langhan's layer of fibrinoid, which in turn was lined by syncytiotrophoblast towards the intervillous spaces. Few

villi were entrapped in this layer and also few extravillous cytotrophoblast cells (X-cells) were seen.

In the placentas of IUGR cases, there were wider regional variations of villous structures. In most of the placentas, syncytiotrophoblastic linings of villi were thinned out with considerable increase in size and numbers of syncytial knots (Fig.3). Capillaries in the villi were increased in number than control placentas, though in some IUGR placentas, intravillous capillaries were less in number, or absent. Fibrin deposits within the villi (*intravillous fibrin deposition*) and between the villi (*perivillous/ intervillous fibrin deposition*) were more in IUGR placentas as compared to those of control placentas. Degeneration of trophoblastic lining of the villi were marked at places, the trophoblastic lining were replaced by perivillous fibrin deposits (Fig. 4).

Perivillous fibrin depositions were marked in the subchorionic areas, as well as near the basal plates. The entrapped villi were sclerosed, lacked syncytial lining and capillaries were either reduced in number or absent (Fig. 5). Areas of *infarction* were found to be extensive in the placentas of IUGR cases, characterized by villous crowding, necrosis of the syncytiotrophoblastic lining, extreme congestion of villi; obliteration of intervillous spaces by a mixture of coalescence of villi and fibrin deposition were also noted (Fig. 5).

Basal plates were noted to have fibrinoid deposits, presence of increased number of extravillous cytotrophoblasts (X-cells), decidual cells, increased number of mononuclear leucocytes, and few giant cells. The *uteroplacental blood vessels* were lined with endothelial cells, and at places, their tunica media were replaced by fibrinoid (Fig. 6).

Langhan's layer of fibrinoid towards the intervillous spaces lined *chorionic plates*. In most of the placentas,

**Table-1 (b):** Showing data regarding counting of syncytial knots and x- cells in control placentas:

number of syncytial knots (control placentas)				number of x-cells in basal plate (control placentas)			
Total no. counted	Maximum in an area of 0.02 25 mm <sup>2</sup>	Minimum in an area of 0.02 25 mm <sup>2</sup>	Average in an area of 0.02 25 mm <sup>2</sup>	Total no. counted	Maximum in an area of 0.02 25 mm <sup>2</sup>	Minimum in an area of 0.02 25 mm <sup>2</sup>	Average in an area of 0.02 25 mm <sup>2</sup>
126	10	5	7	144	12	5	8
108	7	5	6	144	10	6	8
90	5	2	5	198	12	6	11
90	6	4	5	108	12	2	6
126	8	4	6	90	9	2	5
72	5	3	4	126	9	4	7
54	7	4	3	162	10	5	9
90	10	4	5	342	21	14	19
54	7	2	3	90	9	4	5
90	7	4	5	108	8	4	6
72	6	3	4	180	19	6	10
108	7	3	6	162	9	7	9
72	9	3	4	144	13	4	8
90	8	4	5	108	18	5	6
90	10	5	5	162	15	3	9
54	8	2	3	126	12	2	7
90	6	4	5	270	18	9	15
108	8	3	6	162	11	4	9
126	9	5	7	144	10	6	8
72	7	4	4	288	20	9	16
90	7	5	5	108	9	4	6
126	8	4	7	198	15	7	11
108	7	3	6	162	9	7	9
90	10	4	5	342	21	14	19
54	7	4	3	162	10	5	9

**Table-2:** Average numbers of syncytial knots /0.0225 mm<sup>2</sup> area

	mean	max.	min.	p value
control	5	10	2	<0.001
iugr	7	13	3	

the thicknesses of these layers were extensive with a large number of villi entrapped in them. The villi had incomplete syncytial lining and less vascularity. Large numbers of X-cells were also seen entrapped in the fibrin.

The average number of syncytial knots was found to be 5 with a range of 2-10 in the normal-weight or control placentas (Table-1b). The mean number of syncytial knots in IUGR was higher, it was found to be 7, range 3-13 (Table-1a).

The number of X- cells present in a unit area of basal plate was counted. In control placentas, the number ranged from 2- 21 with a mean of 9 (Table-1b). In IUGR placentas, numbers of X- cells ranged from 5- 38, with a mean of 16. (Table 1-a), which was a higher figure.

**DISCUSSION**

On light microscopic study of the histological sections of the placentas of both control and IUGR group, marked regional variations of structures of chorionic villi, the intervillous spaces, basal and chorionic plates were noted.

In the chorionic villi, *syncytiotrophoblastic linings* were thinned out in most of the areas of control placentas, whereas in IUGR placentas, the linings were thinned as well as disrupted. Villous *cytotrophoblast cells* were seen at places along the margins of villi in both the groups. In IUGR placentas, they were more numerous and even present within the villous stroma. Previous studies made similar observations.<sup>5,6</sup>

*Syncytial knottings* were present in both groups, but they were more prominent and their numbers were increased in IUGR placentas. Our findings were in accordance to other workers.<sup>7</sup>

One feature that drew our attention was presence of *intravillous fibrinoid depositions*. Though they were present at places in term control placentas, in IUGR cases they were more frequently observed. Few X-cells were found entrapped in these intravillous fibrinoid. While masses of fibrinoid which replaced villous stroma and vasculature underneath a more or less intact trophoblastic cover had been reported earlier, we did not find any intact trophoblastic lining. Some workers postulated that formation of this intravillous fibrinoid might be due to immunological attack against villous cytotrophoblast.<sup>2</sup> More probably it resulted from antigen-antibody reaction in villous stroma.<sup>8</sup>

We found that in IUGR cases degenerated trophoblastic lining of villi were largely replaced by perivillous fibrin

**Table-3:** Average numbers of x-cells in basal plate (area = 0.0225 mm<sup>2</sup>):

	mean	max.	min.	p value
control	9	21	2	<0.001
iugr	16	38	5	

depositions. Replacement of degenerated trophoblastic linings of villi by perivillous fibrin depositions were positively correlated with IUGR.

The perivillous fibrin depositions were marked in the subchorionic areas as well as near the basal plates in some IUGR placentas. The entrapped villi were sclerosed, lacked syncytial lining and many were avascular. These perivillous fibrin depositions might be acting as a barrier between fetal and maternal circulation, thereby reducing the transfer of the essential nutrients to the fetus, thus causing IUGR.<sup>9,10</sup>

In our study, small areas of infarctions were observed even in the control placentas. In IUGR placentas, these features of infarction were found to be more marked and extensive. Significant incidence of infarction in IUGR placentas were described by others.<sup>11,12</sup>

In the present study, areas of some IUGR placentas showed chorionic villi with increased number of capillaries. This hypercapillarisation and increased syncytial knotting might be induced by hypoxia, as suggested by experiments on animal.<sup>13</sup> We also observed numbers of avascular villi, but their presence was not prominent enough to suggest them to be the main microscopic findings of IUGR placentas, this observation was in contrast with that of other workers.<sup>14</sup>

In placentas of IUGR babies of the present series, *basal plates* did not have typical layering, which was also in agreement with other workers.<sup>15</sup> There were lining of syncytiotrophoblasts in patches in the surface of the basal plates facing the intervillous space. Mostly they were degenerated and replaced by Rohr’s fibrinoid. Then a mixture of X-cells, decidual cells and fibrinoid were noted. Fibrinoids were more extensive in IUGR placentas. No other worker reported such detailed observation.

In our series, the numbers of X-cells were markedly increased in the placentas of IUGR babies. These findings were in accordance with some others.<sup>16,17</sup>

We noted that the *uteroplacental blood vessels* were lined with endothelial cells at places, but in many of them tunica media and adventitia were found to be replaced by fibrinoid. At some places, the large cytotrophoblasts were also seen lying along the margins of the vessels in IUGR placentas. Similar observations were reported by others.<sup>18,19</sup>

We counted the numbers of syncytial knots in 30 fields

per placenta, in an area of 0.0225 mm<sup>2</sup> with a square graticule. The mean numbers of syncytial knots in IUGR placentas was 7, this value was found to be significantly greater than that of the control placentas, value of which was 5 (Table-2). These findings were in agreement with previous workers.<sup>7</sup> This suggested hypoxia and reduced perfusion in cases of IUGR.

Using the same graticule, we counted the numbers of X-cells in 30 fields in 0.0225 mm<sup>2</sup> area of basal plate per placenta. The mean number of X-cells were found to be significantly greater in cases of IUGR placentas (value = 16), as compared to that of the control placentas (value = 9) [Table-3]. Similar reports of increased X cells by eminent workers<sup>4,17</sup> were available, without any statistical analysis. It was proposed that the X-cells were responsible for production of major basic protein (MBP) which was toxic.<sup>4</sup> Increase in number of X-cells might be a cause in IUGR.

Light microscopic findings suggested that the syncytiotrophoblastic linings were more degenerated and number of syncytial knots were increased in IUGR placentas in comparison to those of the control placentas. Increased syncytial knots per unit area might have occurred due to reduced perfusion of villi and decreased utero-placental blood flow. X-cells were more in IUGR and than in control placentas. Cytotrophoblasts possibly became prominent and numerous in the attempt to replace and repair damaged syncytiotrophoblasts in the ischemic placentas.

Intravillous and perivillous fibrin depositions were present in both the varieties of placentas, but were markedly increased in IUGR. Numerous villi were entrapped in the perivillous fibrin deposition, thereby reducing the functioning mass of villi, causing placental insufficiency. In some IUGR placentas, intravillous capillaries were either increased in numbers or in diameters. This might have resulted from hypoxia. In some other IUGR placentas, villi were hypovascular / avascular. Large areas of infarction were present in the IUGR placentas. Placental infarction might be valuable marker of utero-placental vascular disease related to IUGR and impaired fetal and umbilical blood flow.

In basal plates, numbers of X-cells were markedly increased in IUGR placentas. The tunica media of uteroplacental blood vessels were replaced by fibrinoid at places.

Chorionic plates were observed to have multi-layered structure. They were lined by Langhan's layer of fibrinoid, which in turn was lined by syncytiotrophoblast towards the intervillous spaces. This layer was very thick in most of the IUGR placentas.

It was evident that a single placental lesion had not resulted in growth restriction of the fetus. Multiple

factors, e.g. poor perfusion, presence of increased number of X-cells, increased fibrin deposition; etc for a sufficient time interval were likely to have lead to fetal growth restriction.

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