# A COMPARATIVE STUDY OF PLACENTAL VILLOUS CHANGES IN NORMAL AND ABNORMAL PREGNANCIES

by

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*Introduction*

Irrespective of size of dimensions, few obstacles have yet proved unsurmountable before man's indomitable will and persistence. It is this hope alone which sustains our constant efforts to study the mysterious structure of the placenta, and every study, no matter how small contributes towards this goal.

A variety of changes in the morphology of the placental villi in normal and abnormal pregnancies have been reported and also increased deposition of P.A.S. positive, fibrinoid material in the placental villus due to toxaemia of pregnancy (Burstein, 1957). Others Fox (1964) and Wigglesworth (1964) have attributed all changes to a diminished uteroplacental blood flow.

*Materials and Methods*

Placentae were collected from patients delivered in the Obstetric Unit of the Post Graduate Institute, Chandigarh. Cases were divided into two main groups, normal and abnormal.

(1) Patients who were included in normal group (30) had delivered after 38 weeks gestation, had a Hb% above 10 gms % and a normal urine analysis, irrespective of the mode of delivery.

The Abnormal Group was further divided into, (I) anaemic, (II) toxaemic, (III) postmature or (IV) Rh. incompatible blood groups.

(I) Cases in the anaemic Group (30) had Hb values below 10 gms. %, showed no signs of toxaemia. Toxaemic group (15) included patients who had hypertension and/or oedema, albuminuria, but had a haemoglobin level above 10 gms. % (III) Postmaturity group (4) consisted of patients whose gestational period was 42 weeks and above but were otherwise normal. (IV) Rh. and ABO incompatibility groups (4) comprised cases detected in the antenatal period, and subsequently treated and followed through to delivery with antibody titres, etc.

*Collection of Placentae*

Placentae were collected immediately after delivery, labelled and immersed in 4% formaldehyde solution for at least 10 days. The placentae were blocked, sectioned and processed after the method described by Fox (1964).

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Four sections were selected at intervals of 100 $\mu$ for study and were stained with different stains.

(1) Haematoxylin-eosin, for general scrutiny, study of syncytial knots vasculosyncytial membranes and hypovascularity. (Fig. I)

(2) Van Geison stain was used for the study of collagen (stromal fibrosis). (Fig II)

(3) Alcian blue, paramino salicylic acid for mucopolysaccharides. (Fig. III)

(4) Mallory's P.T.A.H. staining was used for identification of fibrin deposits. (Fig. IV)

The sections were stained and examined as suggested by Fox (1964).

*Results*

Table I presents the percentage of placentae and their corresponding percentage counts of the various characteristics, derived at from counts of 400 villi in each parameter. The values of each parameter were statistically analysed as shown in Table II.

*Discussion*

Each characteristic is discussed below:—

*Syncytial Knots*

The counts of the syncytial knots were found to range between 30-50% in majority of normal cases. This finding concurred with the work of Merrill (1963). However, Benerische (1961), Fox (1965) and Malkani and Bhasin (1968) suggested an excess of over 30% count to be indicative of excessive ageing due to either

TABLE I

*Percentage of Placentae in the Various Groups and Their Corresponding Counts Per 100 Villi*

| S. No. | Parameter counts per 100 villi | Normal (30) percentage placentae | Anaemia (30) percentage placentae | Toxaemia percentage placentae | Rh & ABO incompatibility percentage placentae | Postmaturity percentage placentae |
|---|---|---|---|---|---|---|
| 1. Syncytial knots | less than 30%, | 23 | 7 | 7 | 0 | 0 |
| | 30-50%, | 73 | 83 | 73 | 72 | 75 |
| | above 50% | 4 | 10 | 20 | 28 | 25 |
| 2. Vasculo-syncytial membranes | less than 60%, | 10 | 0 | 0 | 0 | 0 |
| | 6-30%, | 73 | 66 | 86 | 85 | 75 |
| | more than 30% | 17 | 34 | 14 | 15 | 25 |
| 3. Hypovascular villi | less than 4%, | 63 | 60 | 73 | 57 | 25 |
| | 4-10%, | 37 | 33 | 20 | 28 | 75 |
| | more than 10% | 0 | 7 | 7 | 15 | 0 |
| 4. Stromal fibrosis | less than 3%, | 23 | 4 | 1 | 14 | 0 |
| | 3-7%, | 70 | 83 | 70 | 71 | 75 |
| | 7-10% | 7 | 13 | 20 | 15 | 25 |
| 5. Thickening of basement membrane | less than 3%, | 30 | 10 | 20 | 57 | 25 |
| | 3-5%, | 57 | 20 | 40 | 28 | 0 |
| | more than 5% | 13 | 70 | 40 | 15 | 75 |
| 6. P.A.S. positive villi | less than 3%, | 16 | 10 | 13 | 14 | 0 |
| | 3-6%, | 80 | 67 | 53 | 43 | 0 |
| | more than 6% | 4 | 23 | 34 | 43 | 100 |
| 7. Fibrinoid (P.T.A.H.) | less than 3%, | 40 | 20 | 26 | 14 | 0 |
| | 3-6%, | 44 | 17 | 40 | 14 | 40 |
| | more than 6% | 16 | 63 | 34 | 72 | 60 |

TABLE II
*Statistical Analysis of Results*

| S. No. | Parameter | Normal (30) Mean | S.D. | Toxaemia (15) Mean | S.D. | Probability level (P) | RH & ABO Incomp Mean | S.D. | Probability level (P) | Post-maturity (4) Mean | S.D. | P | Anaemia (30) Mean | S.D. | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Syncytial Knots | 140.80±28.46 | | 168.33±39.76 | | <0.05 | 192.87±56.61 | | <0.05 | 164.00±30.85 | | >0.05 | 147.20±30.24 | | >0.05 |
| 2. | Vasculo-Syncytial membranes | 91.57±29.08 | | 97.47±23.74 | | >0.05 | 99.28±16.67 | | >0.05 | 119.10±40.64 | | >0.05 | 104.80±34.56 | | >0.05 |
| 3. | Hypovascular villi | 12.70± 4.41 | | 14.67± 9.77 | | >0.05 | 18.71±14.70 | | >0.05 | 17.50± 6.13 | | >0.05 | 14.80±13.36 | | >0.05 |
| 4. | Stromal fibrosis | 17.60± 8.03 | | 26.53±11.77 | | <0.05 | 25.71±15.60 | | >0.05 | 33 25±11.44 | | <0.05 | 27.20±10.12 | | <0.01 |
| 5. | Fibrin like deposits P.T.A.H. | 16.90±12.20 | | 28.20±30.36 | | >0.05 | 36.71±21.80 | | >0.05 | 29.50±16.34 | | >0.05 | 23.28±11.20 | | <0.05 |
| 6. | Thickening of basement membrane | 16.13± 8.25 | | 19.00± 9.24 | | >0.05 | 11.00± 6.78 | | >0.05 | 21.00±10.61 | | >0.05 | 31.20±15.32 | | <0.01 |
| 7. | P.A.S. positive villi | 15.93± 6.16 | | 18.67± 7.43 | | >0.05 | 18.71± 8.12 | | >0.05 | 14.50± 1.73 | | >0.05 | 16.80± 6.05 | | >0.05 |

1. The mean and standard deviations are based on counts of 400 villi in each characteristic.
2. The value of the statistic 'T' thus obtained has been compared with 't' at 5% level, meaning probability level P at 0.05.
3. The figures in parenthesis indicate the number of cases in each group.

postmaturity or a disease state causing placental insufficiency.

The mechanism of syncytial knot formation although yet disputed appears to favour the postulations of Fox, who claimed their proliferation to be due to an amniotic division of the syncytial knots' nuclei. Others, however, suggested its formation to indicate ischaemic response or functional inactivity (Thomsen 1965) and Getzova and Sadowasky (1967).

In toxaemia and Rh. and A.B.O. incompatibility the counts of syncytial knots were significantly high ($P<0.05$, Table II).

*Vasculo-Syncytial Membranes*

These membranes characterise the mature placental villi. The function of this thinned out, apparently anuclear membrane is predominantly a passive filtration (Amstutz, 1960). Most of the normal cases (73%) had counts in the range of 6-30%, similar to those reported by Fox (1967). Observations in this study showed no consistent relationship between the vasculo-syncytial membrane counts and the period of gestation. Moreover, there was no significant variation in these counts in response to decreased oxygen supply (Horky, 1964). There was also no significant decrease in vasculo-syncytial membrane counts, in cases associated with foetal distress or asphyxia at birth or even if the baby was stillborn.

*Hypovascular and Avascular Villi*

The various postulations for the production of hypovascularity include period of gestation, placental ischaemia and foetal anaemia (Badarau, 1967 and Fox, 1967). These, however, have failed to explain fully the mechanism involved. The popular theory of a steady continous fresh growth of villi is a plausible explanation for the presence of both the large immature looking hypovascular villi, as also the typically "regressive" villi.

However, a predominance of one or the other variety of hypovascularity would indicate the period of gestation or the approximate age and functional status of the placenta.

The counts in the present study were neither indicative of any particular disease state nor statistically significant.

*Stromal Fibrosis*

It is probable a number of factors may contribute to the formation of stromal fibrosis. In this study, about 93% of the normal cases showed less than 7% stromal fibrosis. The figures given by Fox (1968) are, however, lower (3%).

In the toxaemic group, 20% of the placentae showed marked stromal fibrosis (more than 10%) as also 13% of the anaemic group. The Rh. incompatibility group showed moderate fibrosis (6-10%) in 28% of the placentae, although almost 71% had mild fibrosis (3-6%). Even in the postmature group, stromal fibrosis of more than 10% was observed in 25% of the cases. The two main factors thought to be responsible for the formation of stromal fibrosis are, normal ageing process and a reduced uteroplacental blood flow. The first is, however, inconclusive in that stromal fibrosis of marked degree has also been observed in prematurely delivered placentae. The second theory supported by Burstein, Blumenthal and Soule (1951) attribute stromal fibrosis to a reduced villous blood flow, due to endarteritic changes in the vessels. This, however, is refuted by Becker and Bleyl (1961) and Kubli and Budlinger (1968).

Our observations in the anaemic group where there was stromal fibrosis without any demonstrable endarteritic changes

supposes that a relative anoxia may be the main factor operative in its production. Higher incidences of stromal fibrosis had no deleterious effect on the fetus as the condition of the baby was uniformly good at birth.

*Basement Membrane Changes*

An undue thickening of the basement membrane (more than 5%) was found in 40% of the placentae of the toxaemic group. More significant however, was the finding of undue thickening of the basement membrane in more than 70% of the placentae in the anaemic group. The normal level for thickening of the basement membrane was taken as 3-5%, based on the finding of this level in 86% of the placentae in the normal group. This is in agreement with the figures given by Fox (1968). Thickening of the basement membrane is said to occur as a result of placental ischaemia. Another important association was the finding of a noticable cytotrophoblastic proliferation in these cases. However, there was no increased thickening of basement membranes in our small group of Rh. incompatibility, as was observed by other workers (Burstein 1963).

*Fibrinoid Necrosis*

A quantitative study by Fox (1968) estimated less than 3% of P.A.S. positive villi as the limit of the normal. Our estimate as counted in P.A.S. and P.T.A.H. stained slides was about 6%. This was based on the finding of this figure in 96% and 84%, respectively by the two methods in the normal group. Of these only 16.6% showed less than 3% of P.A.S. positive villi 42% of Rh incompatible group, 34% of the toxaemic group and 23% of the anaemic group showed more than 6% fibrinoid material. These observations suggested an immunological cause as the basis, as this reaction was exaggerated in conditions of blood group incompatibility and toxaemia of pregnancy. Current evidence suggests that fibrinoid is a complex of heterogenous nature varying to a great extent with the circumstances provocative of its deposition. Wislocki and Bennet (1943), and Fox (1968) are of the opinion that it represents a degenerative change. Kline (1951) suggests that the fibrin is derived from the foetal blood in the villus capillaries; Mckay (1958) and Wigglesworth (1964) attributed to a deposition of fibrin from the maternal blood.

*Staining with P.T.A.H.*

With this method, 34% of the toxaemic group and 63% of the anaemic group showed fibrinoid in more than 6% of the terminal villi. This was found to be statistically highly significant $P>0.05$ (Anaemia). This stain defines fibrin-like deposits clearly inside the villous core and is superior to the P.A.S. method in which it is sometimes difficult to differentiate between intervillous deposits and lesions inside the villi.

*Summary*

Eighty-six placentae were examined histologically and the parameters used, the observations and statistical analysis presented.

Statiscally significant high counts for syncytial knots were observed in toxaemia and Rh incompatible group. ($P<0.05$). Significantly high values were found in counts of stromal fibrosis in toxaemia and postmature cases at 5% level of significance ($P<0.05$).

In anaemic cases the level of significance of stromal fibrosis was 1% ($P<0.01$). Highly significant increase in fibrin-like deposits was observed in

anaemia (P.T.A.H.) ($P < 0.05$). Thickening of the basement membrane was also observed in anaemic group at a statistically significant level of 1% ($P < 0.01$).

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*See Figs. on Art Paper I*