Original Article

Histopathological Study of Villous Morphology in Spontaneous First Trimester Abortions

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ABSTRACT

Background: First trimester abortions are seen in 10 – 20 % of pregnancies. Sending the tissue evacuated after miscarriage for histopathological examination is a topic of debate till date, as some professionals feel it is a waste of time and expensive, while on the other hand a school of thought still persists on doing the same.

Methods: We studied the various histopathological changes seen in the abortus tissue in first trimester spontaneous abortions over a period of over a period of one year. A total of hundred slides and requests for histopathological examination of first trimester abortions were retrieved and studied in detail by two pathologists for the following features- Villous size, contour, vasculature, trophoblastic proliferation, perivillous and intervillous hemorrhage, perivillous fibrin deposition, stromal fibrosis, inflammation and decidual change. The observed changes were also categorized according to the weeks of abortion. Appropriate statistical tests were employed.

Results: Our study showed many dysmorphic features in the villi like reduced vessels per villous (72%), fibrosis (21.3%), hydropic change (32%) and abnormal trophoblastic proliferation (49%). Other features noted were villous hydrops, inter and perivillous fibrin deposition, inflammation, decidual change and Arias Stella reaction. Reduced vessels per villi and hydropic change were significantly associated with abortions happening at 8 - 10 weeks, while reduced patency of vessels, abnormal villous contour, ghost villi and trophoblastic proliferation were more associated with earlier dated abortions.

Conclusion: Cases with dysmorphic features as seen in the present study are known to be associated with clinically significant conditions like diabetes, eclampsia and with certain chromosomal abnormalities. Such cases can not only be filtered for cytogenetic work up, but documentation of these features can also aid in counselling and planning of future pregnancies. Thus histopathological examination of abortus material is highly recommended.

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Introduction

The commonest complication of pregnancy is abortion, its incidence being around 15%.[1] Though genetic aberrations are associated with higher frequency of spontaneous abortions the exact etiology is still unknown.[2] The development of fetus and placenta occurs simultaneously and hence fetal developmental abnormalities will be reflected with the changes observed in the foetal part of the placenta.[3] This aberrant growth of the placenta is recognized by the uterus as abnormal and thus it expels such products giving rise to spontaneous abortions. Thus the study of histological aspects of chorionic villi in spontaneous abortions may be imperative in understanding the etiology and pathogenesis of abortions.[4] Our study aims to explore the various histological changes occurring in the chorionic villi in first trimester abortions.

Materials and Methods

Around 100 cases of curetted material of first trimester abortions sent for histopathological examination were studied from January 2015 to December 2015. Relevant clinical history was obtained from the requisition forms sent along with the curetted material. The specimens underwent routine histopathological processing and were stained with Haematoxylin and Eosin stains. Special stains were employed wherever necessary. The slides were examined by two pathologists independently.

The following histological parameters suggestive of villous maldevelopment or degeneration along with a few other features were studied and categorized. [5, 6]

1. Villous size- small, intermediate, large
2. Villous contour – round, irregular, scalloping
3. Villous vasculature **Grade I:** normal (**8- 10vessels** per villous). Vessels with nucleated blood cells are present in almost every (at least nine of 10) villus, have a very clear appearance and are located centrally as well as peripherally (in contact with the trophoblastic layer). In some villi, the number of vessels are even numerous (>5).

   Grade IIA: mild hypoplasia (**5 – 8 vessels** per villous). Vessels with nucleated blood cells are not present in all villi, less numerous and predominantly located centrally.

   Grade IIB: severe hypoplasia **(3 – 4 vessels** per villous). Villi are predominantly avascular, however, in a single villous, a vessel is present with one or more nucleated blood cells.

   Grade III: avascular **< 3 vessels** per villous. All villi are avascular, although sporadically a very small vessel, with or without a nucleated blood cell may be present.[4]
4. Trophoblastic proliferation
5. Perivillous and intervillous haemorrhage
6. Perivillous fibrin deposition – none , few , intermediate, abundant,
7. Ghost villi
8. Hydrops : marked villous edema > 25% of villi
9. Stromal fibrosis: grade 1 < 6 % grade II > 6%
10. Hoffbauer macrophages
11. Arias Stella Reaction
12. Decidualised tissue
13. Inflammation

Exclusion Criteria: Curetted samples of spontaneous first trimester abortions, having risk factors and /or associated or with proven morbid conditions like diabetes, hypertension, infections etc were excluded from the present study.

Statistical Analysis: Descriptive and inferential statistical analysis was carried out in the present study. Results on continuous measurements were presented as Mean ± SD (Min-Max) and results on categorical measurements were presented in Number (%). Significance was assessed at 5% level of significance. P value < 0.5 was considered significant. The following assumptions on data were made.

Assumptions

1. Dependent variables are normally distributed.
2. Samples drawn from the population are random. Chi-square/Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups.

Statistical Software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel were used to generate graphs, tables etc.

Results

A total of 100 cases were retrieved, of which 25 were excluded. The cases excluded were those of voluntary medical termination of pregnancy (MTP), cases which on microscopy showed no villi, and which had areas of extensive hemorrhage and necrosis to the extent of obscuring the morphology.

The age group of the females ranged from 18 to 37 years, with 77.3% falling in the 20 – 30 age group. The types of abortions were as categorized as follows [Table 1].

The patients were categorized on the basis of weeks of abortions as follows.

Histopathology: Sizes of majority of the villi were intermediate (47%) with large villi seen in 17.3% of the cases and the rest being small sized villi.[Figures 1A, 1B and 1C].

Regular villous contour was seen in 54.7% of the villi. Ghost villi were seen in 50.7% with hydropic change in 32% of the cases. Increased trophoblastic proliferation was seen in 49% of cases. Decidual change was seen in 53.4% of cases. Significant stromal fibrosis was seen in 23.5% of the cases.

Severe inflammatory reaction comprising of a mixture of neutrophils along with lymphocytes and plasma cells were seen in 5.9% of the cases. Ghost villi or hyalinized villi were seen in 50.7% of the cases. The various morphological features were compared with the weeks of abortion [Table 3 and Table 4].

Surprisingly, 72 % of the villi had less than 3 vessels per villi. Intervillous haemorrhage was seen in 44 % of the villi. A total of 21.3% of the villi showed abundant fibrin deposition. [Fig 2A and 2B]. The fibrin deposition was further highlighted using Masson's Trichome stain.

When categorized the changes according to the weeks of abortion it was seen that reduced vessels per villi and hydropic change [Fig 8A] were predominantly features of abortions seen during 8 – 10 weeks, while reduced patency of vessels, abnormal villous contour, ghost villi and trophoblastic proliferation were predominantly seen in earlier dated abortions. However, there was no statistically significant co- relation between the weeks and variables. Other features observed were increased Hoffbaeur cells [Fig 8B] in about 34.6% of cases, Arias -Stella reaction, decidualised tissue and endometrial glands**.**

Table 1: Types of abortion.

Abortion type	%
Incomplete Abortion	88
Missed abortion	9.3
Inevitable abortion	2.7
Total	**100**

TABLE 2:Categorization of the abortion according to weeks:

Weeks of abortion	%
<6	22.7
6-8	36
8-10	28
10-12	13.3
Total	**100**

Table 3: Correlation of weeks of abortions and study morphological variables.

Variables	No. of patients (n=75)	Weeks of Abortion				
		<6.0 (n=17)	6.0-8.0 (n=27)	8.0-10.0 (n=21)	10.0-12.0 (n=10)	P value
Size (mm)						
• Intermediate	47(62.7%)	9(52.9%)	14(51.9%)	17(81%)	7(70%)	**0.21**
• Large	13(17.3%)	2(11.8%)	6(22.2%)	3(14.3%)	2(20%)	
• Small	15(20%)	6(35.3%)	7(25.9%)	1(4.8%)	1(10%)	
Vessels per Villi						
• 8-10	2(2.7%)	-	-	2(9.5%)	-	**0.05**
• 5-8	-	-	-	-	-	
• 3-4	19(25.3%)	5(29.4%)	9(33.3%)	4(19%)	1(10%)	
• <3	54(72%)	12(70.6%)	18(66.7%)	15(71.4%)	9(90%)	
Contour						
• Nil	1(1.3%)	1(5.9%)	-	-	-	**0.7**
• Regular	41(54.7%)	8(47.1%)	14(51.9%)	13(61.9%)	6(60%)	
• Scalloped	33(44%)	8(47.1%)	13(48.1%)	8(38.1%)	4(40%)	

Variables	No. of patients (n=75)	Weeks of Abortion				
		<6.0 (n=17)	6.0-8.0 (n=27)	8.0-10.0 (n=21)	10.0-12.0 (n=10)	P value
Haemorhage						
• Perivillous	17(22.7%)	5(29.4%)	6(22.2%)	4(19%)	2(20%)	**0.6**
• Intervillous	33(44%)	8(47.1%)	11(40.7%)	10(47.6%)	4(40%)	
Fibrin Deposits						
• Nil	2(2.7%)	-	1(3.7%)	-	1(10%)	**0.4**
• Fewer	21(28%)	6(35.3%)	7(25.9%)	5(23.8%)	3(30%)	
• Intermediate	24(32%)	3(17.6%)	10(37%)	9(42.9%)	2(20%)	
• Abundant	16(21.3%)	3(17.6%)	5(18.5%)	6(28.6%)	2(20%)	

Table 4: Correlation of weeks of abortions and other findings.

Other findings	No. of patients (n=75)	Weeks of Abortion				
		<6.0 (n=17)	6.0-8.0 (n=27)	8.0-10.0 (n=21)	10.0-12.0 (n=10)	P value
Ghost Villi						
• Nil	37(49.3%)	10(58.8%)	11(40.7%)	11(52.4%)	5(50%)	0.7
• +	38(50.7%)	7(41.2%)	16(59.3%)	10(47.6%)	5(50%)	
• ++	-	-	-	-	-	
Hydropic change						
• Nil	51(68%)	11(64.7%)	20(74.1%)	14(66.7%)	6(60%)	0.6
• +	24(32%)	6(35.3%)	7(25.9%)	7(33.3%)	4(40%)	
• ++	-	-	-	-	-	
Stromal Fibrosis						
• Nil	30(40%)	13(76.5%)	10(37%)	4(19%)	3(30%)	<0.001
• <6%	21(28%)	3(17.6%)	13(48.1%)	2(9.5%)	3(30%)	
• >6%	24(32%)	1(5.9%)	4(14.8%)	15(71.4%)	4(40%)	
Decidual change						
• Nil	35(46.7%)	5(29.4%)	11(40.7%)	11(52.4%)	8(80%)	0.5
• +	38(50.7%)	12(70.6%)	15(55.6%)	10(47.6%)	1(10%)	
• ++	2(2.7%)	-	1(3.7%)	-	1(10%)	

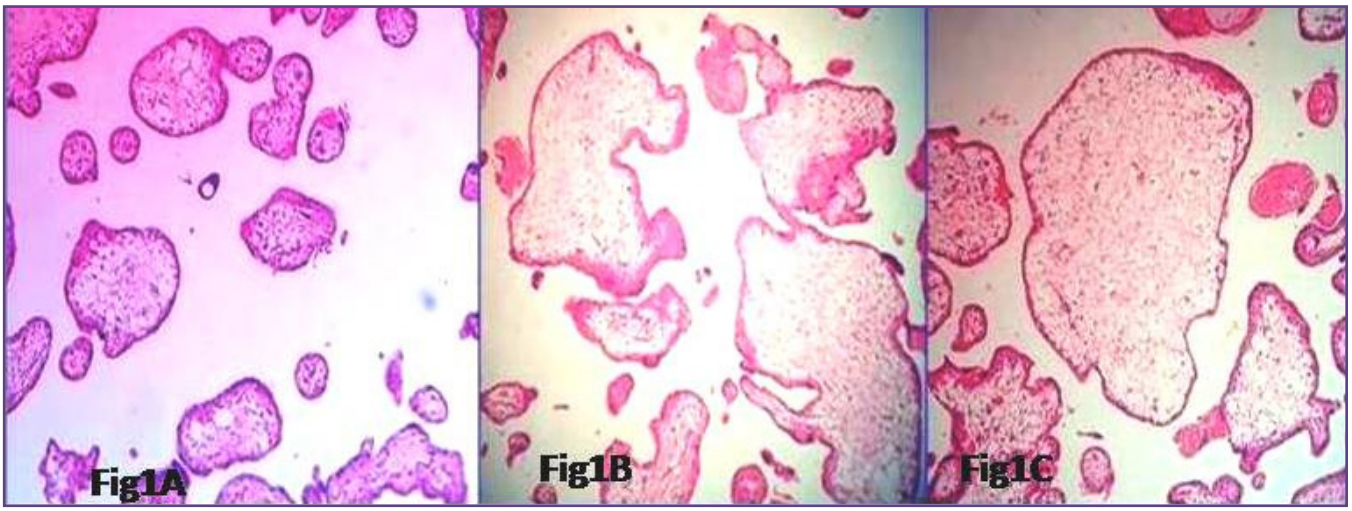

Fig. 1: Microphotography showing varying sized villi a) small b) medium and c) large (H&E, X100).

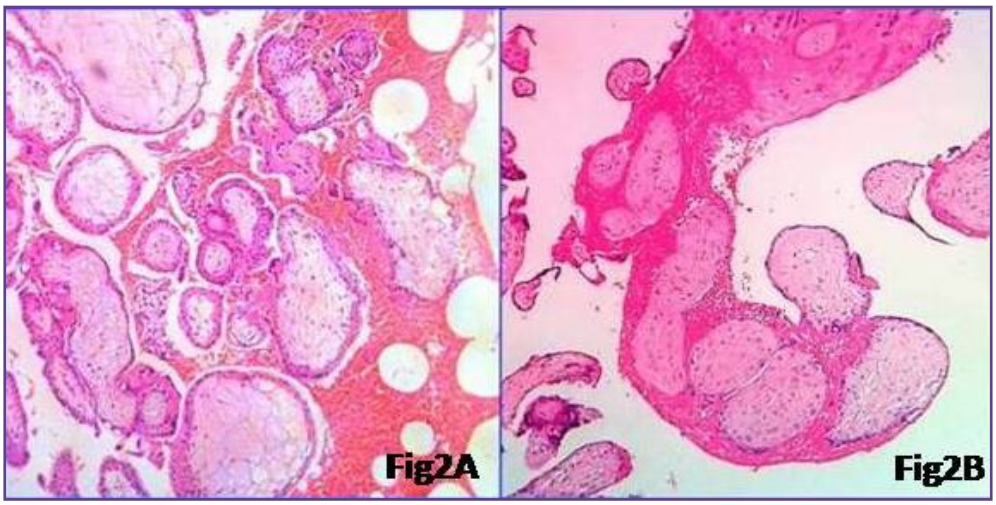

Fig. 2: Microphotograph showing a) intervillous hemorrhage b) perivillous fibrin deposits (H&E,X100).

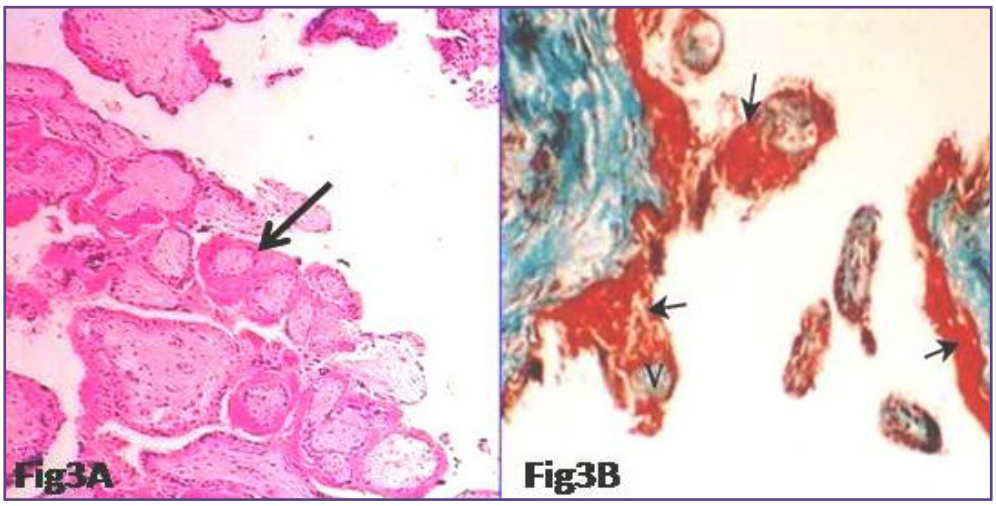

Fig. 3: Microphotograph showing fibrosis- arrows a) (H&E, X100) b) Masson's trichome stain (MTS, X100).

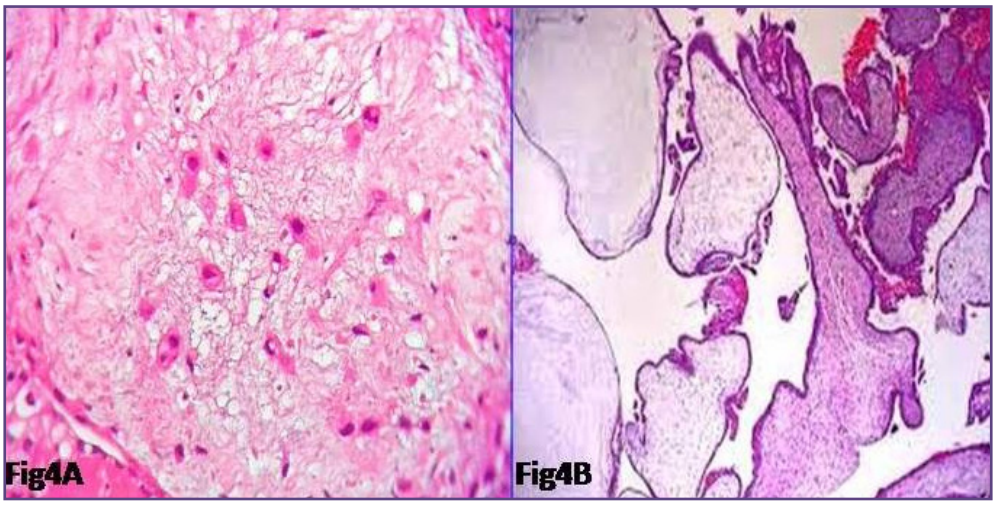

Fig. 4: Microphotograph showing a)Hofbauer macrophages b) large hydropic villi (H&E,X100).

Discussion

An early pregnancy loss or first trimester miscarriage is the most common complication of human reproduction, with an incidence ranging between10 - 20 % of all conceptions. [5, 6]

In most centers it is a routine practice to send the tissue obtained from uterine evacuation after miscarriages for histopathological examination, not only for confirmation of pregnancy but also for medico legal purposes and for ruling out ectopic and molar pregnancies which necessitate special follow up.[5] However till date both school of thoughts pertain, stating the necessity and no necessity of histopathological examination following abortions.

The main morphological villous criteria used to investigate early pregnancy loss are regularity of the villous contour, stromal oedema or fibrosis, foetal vasculature and intervillous fibrin deposition.[6] Irregular villous contour with small villous size are often related to trisomies. In the present study also small villi were seen in 20% of the cases.[7] Large bulbous villi are characteristic of first trimester abortions. Hydropic villi, poorly vascularised villi, villous haemorrhages and increased syncitial knotting points to abnormal villous shapes, usually found under hypoxic conditions.[8]

Although perivillous fibrin is common, intravillous fibrin is abnormal, seen in diabetic pregnancies. [8] Fibrosis is a final common pathway for nearly all forms of diseases that progress towards end organ failure. The resident cells can often stimulate factors producing basement membrane material and cellular matrix molecules like collagen type I, II and 1V fibronection and proteoglycans. [9] Fibrosis is a feature associated with pre- eclampsic and diabetic pregnancies. Edematous villi can be seen in infections like syphilis, CMV and a variety of cases of hydrops. Extensive stromal fibrosis can be seen in cases of intra uterine growth retardation. [8]

Cross section of the vessels in first trimester villi on an average showed 8 – 10 vessels per villous. The cases in our study showed significantly reduced number of vessels per villous.

The reduced villi may be either due to their de novo poor formation or secondary to the fibrosis as mentioned above. Also vascular compromise is a key factor in contributing to miscarriage. Studies have documented the differences in vascularity between villi of spontaneous versus induced abortions. [9] The poor villous vasculature can also explain the stromal oedema, which results from accumulation of water normally drained by the trophoblast before it becomes non – functional. [6] Vascular compromise was a key feature in our study. Vascular changes in first trimester chorionic villi are due to defective vasculogenesis than due to post-mortem changes as proven by studies done by Hakvoort et al.[10]

Reduced vessels per villi was the most consistently seen feature in our study also. A detailed study of the role of Hoeffbaeur cells in abortions have documented their increase in number in cases of missed abortions.[11] The limitation of our study to count the exact number of macrophages was that we have not employed the immunohistochemical marker CD68. Their identification was only based on the routine Hand E stain

Cytogenetics and Villous Morphology

Chromosomal abnormalities are known to be associated with abortions. Trisomies are related to smaller villous size, reduced capillaries per villi and increased perivillous fibrin deposition Villous hydrops is also associated with trisomy. [7] But cytogenetic evaluation of miscarriage specimens is expensive and not always successful. [10] A study on karyotyping of abortus villi by Genest et al [7] has proved that although spontaneous first trimester abortions do have abnormal histomorphological features, not many were diagnostic of a particular abnormal karyotype. [12]

In our study we have observed that some morphological features were seen consistently in all abortions like reduced villous size, compromised vasculature and stromal fibrosis. Studies have proven that dysmorphic villous features although not specific, can still be indicators of chromosomal abnormalities. Although histopathological examination may not be mandatory, documentation of these abnormal features may filter cases for cytogenetic evaluation and other special work up.

Specimens of recurrent abortions have features of chronic inflammation. These many have associated genetic and also non genetic abnormalities like abnormalities in tryptophan metabolism. Factor V mutation and Antiphospholipid antibody syndromes.[13] Chronic intervillositis is associated with recurrent foetal demise.[14]

Histopathological examination of abortions achieves following goals--confirmation of an intrauterine pregnancy, exclusion of hydatidiform mole, recognition of phenotypic clues that warrant a complete cytogenetic study.[7] Review of 670 cases at Charing cross hospital of London , Pathology department showed 120 (18%) of patients with unsuspected molar pregnancy were diagnosed as moles on account of their abundant trophoblast in early pregnancy or by presence of hydrops, thereby favoring histopathological examination of the abortus material.[15]

However till date here is no general agreement about the value of submitting tissue for histopathological examination [HPE]. One explanation of poor predictive value of histology is because the features assessed are variable and there is poor inter and intra observer reproducibility. Histopathological features of partial hydatidiform mole overlap with spontaneous abortions harbouring trisomies. [12] Another postulated reason for poor predictive value of histology would be the retention time of products in the

uterine cavity for about 3 -8 weeks after foetal demise, leading to post mortem artifacts. [11,12]

Study by Heath et al recommended HPE only when pre -operative diagnosis was uncertain or trophoblastic tissue was seen during evacuation. [16] In Asia the incidence of Hydatidiform mole is as high as 1 in 80 pregnacies, unlike the western countries, highlighting the importance of a routine HPE of the abortus tissue.

Recommendations

The cytogenetic analysis of spontaneous first trimester abortions, showing the above mentioned dysmorphic features on histopathological examination will be taken up as the next part of the study after obtaining the patients consent.

Conclusion

Our study has analyzed many dysmorphic villous features which may be the effect or the cause of the first trimester abortions**.** Histopathological examination of abortus material not only confirms the documentation of pregnancy, but can also pick up unsuspected molar pregnancies, and gives clue to associated with clinical conditions like diabetes and eclampsia. Various dysmorphic features like small villous size and reduced vessels per villi, helping to filter the cases for cytogenetic evaluation, which on the other hand is expensive and not easily affordable by patients in a developing country like ours. More standardization is required on the aspect of submitting the aborted material for histopathological examination.

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Competing Interests

None Declared

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