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# HISTOLOGY AND CYTOGENETIC STUDY OF CHORIONIC VILLUS TISSUE IN FIRST TRIMESTER ABORTION

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## ABSTRACT

Pregnancy loss is a very common entity observed in obstetric practice.Most commonly it occurs in first trimester abortions. In this study we are doing histological and cytogenetic analysis of chorion villus tissue in first trimester abortion. This will help us to know whether the pregnancy loss was due to chromosomal abnormality. All the selected cases were in the age group 19-40 years. Abnormal karyotype was found in 38% of the cases. Trisomy was predominated followed by polyploidy, monosomyX, structural anomalies and other abnormality. Regarding histology, the highest precision in categorizing abnormal abortion reached with trisomy and triploidy. It was seen that placental microscopic study alone was not enough to give a proper aeitological diagnosis in case of spontaneous abortion. In conclusion cytogenetic analysis is necessary for differentiating between choromosomally normal and abnormal abortion for further management of the patient.

**KEYWORDS: First trimester abortion, Cytogenetics, Chromosomal anomalies, Chorion villus tissue.**

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# INTRODUCTION

Human development begins at fertilization with the formation of diploid zygote. This unicellular zygote undergoes numerous mitotic divisions followed by cell migration, growth and differentiation to form a multicellular human being[1]. During the 1st week as the zygote passes along fallopian tube towards the uterus it undergoes series of mitotic divisions to form blastocyst. This blastocyst consist of embryoblast and trophoblast. Embryoblast gives rise to embryo proper and trophoblast forms placenta and fetal membranes. During the 2nd week the embryoblast differentiates into epiblast and hypoblast forming bilaminar disc. Simultaneously trophoblast differentiate into inner cytotrophoblast and outer syncytiotrophoblast. Cytotrophoblast forms cellular columns surrounded by syncytiotrophoblast forming primary villi. In the 3rd wk- bilaminar disc becomes the trilaminar embryo. There is formation of secondary villi followed by tertiary villi. During 4th -8th wk all the three layers give rise to its own tissues and organ system. Simultaneously a complex vascular network is established in the placenta by the end of 4$^{th}$ week, which facilitate maternal embryonic exchange of gases, nutrients & metabolic waste products. Growth and thickness of placenta continues rapidly until fetus is of 18 weeks old[2]. Placenta becomes functional from 6-8 weeks of pregnancy and is a vital organ for maintaining pregnancy and promoting normal foetal development[3]. There are many factors that are responsible for a successful pregnancy and any alteration in them can lead to pregnancy loss. Pregnancy loss most commonly occurs in first trimester of pregnancy. The incidence of first trimester pregnancy loss ranges between 50-70 % of all conceptions. First trimester pregnancy loss are related to maternal and fetal factors. 1) Maternal factors include (A) anatomical abnormalities (10-15%). It could be congenital uterine anomalies like bicornuate, unicornuate & septate uterus. Acquired defects like cervical incompetence, uterine fibroid and intrauterine adhesions. (B) Infection (5%) can also cause early as well as late abortion. Infection could be viral like rubella, cytomegalovirus; parastic - toxoplasma, malaria; bacterial-Chlamydia, L. monocytogenes. (C) Endocrine factors (10-15%) comprises luteal phase defect, thyroid abnormalities and diabetes mellitus. (D)Immunological disorders (5-10%) like autoimmune and alloimmune causes miscarriage (E) Hematological factors such as thrombophillic defects, activated protein c resistance can also lead to pregnancy loss. (F) Environmental causes like smoking and excessive alcohol consumption. 2) Fetal causes include Chromosomal abnormalities which account for 50% of all Spontaneous miscarriage. Chromosomal abnormalities are of two types Numerical and Structural abnormalities a) Numerical abnormalities are Aneuploid which includes Monosomy, Trisomy, Tetrasomy and Polyploidy which include Triploidy and Tetraploidy. B) Structural abnormalities are Translocations which is of two types Reciprocal and Robertsonian; Deletion, Insertion, Inversion[4,5,6]. In this study we are doing histological and cytogenetic analysis of chorion villus tissue in first trimester abortion. This will help us to know whether the pregnancy loss was due to chromosomal abnormality. It helps clinicians in management of next pregnancy.

# MATERIALS AND METHODS

50 cases of first trimester abortion in 1st - 13 weeks of gestation were selected for the study. All the selected cases were in the age group of 19 to 40 years. Detail clinical and family history was recorded for all the cases. Obstetric history was taken in the form of pedigree chart. Histology and cytogenetic analysis from all the 50 samples of products of conception was performed by implementing standard protocol of Planting, Harvesting, Banding, and Screening. Products of conception samples were collected in RPMI1640 culture medium and transported to the laboratory. Clean sample was dissected and chorion tissue was separated. The tissue was cultured in amniomax medium for 10-14 days. Samples were then harvested using trypsin EDTA, KCL and fixative (3:1 methanol acetic acid) and karyotypes were prepared. All the tissues were

then subjected for histological study. Tissue was fixed in formalin for preparation of histological slides. Then tissue processing and Haemotoxylin and eosin staining was done according to standard protocol.

## RESULTS/OBSERVATIONS

**Table 1**
***Gestation age and frequency of chromosomally abnormal abortions.***

| Week of Gestation | Chromosomally abnormal abortions | Chromosomally abnormal abortions (%) |
|---|---|---|
| 6-7 | 3 | 6 |
| 8-9 | 7 | 14 |
| 10-11 | 6 | 12 |
| 12-13 | 3 | 6 |
| **Total** | **19** | **38** |

**Graph 1**
***Gestation age and frequency of chromosomally abnormal abortions (%).***

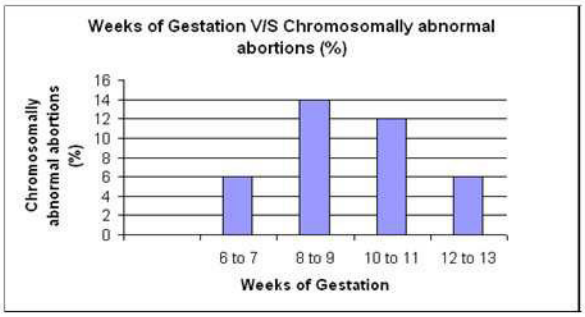



**Table 1**
***Summarize the relation between gestational age and frequency of chromosomally abnormal abortion. Chromosomally abnormal abortion were seen maximum between 8 to 9 week of gestation and were found to be 14% of total cases.***

**Table 2**
***Karyotype of abortions and number of abortions.***

| Karyotype of Abortions | No. of Abortions | % of no. of abortions |
|---|---|---|
| Normal | 31 | 62% |
| Abnormal | 19 | 38% |
| Polyploidy | | |
| 3n | 2 | |
| 4n | - | 21.1% |
| 2n/4n | 1 | |
| 2n+1/4n | 1 | |
| Trisomy | 9 | 47.4% |
| Monosomy X | 3 | 15.8% |
| Structural anomaly | 1 | 5.2% |
| Double cell line | 2 | 10.5% |
| Total | 50 | |

**Graph 2**
***Karyotype of abortions and No. of abortions***

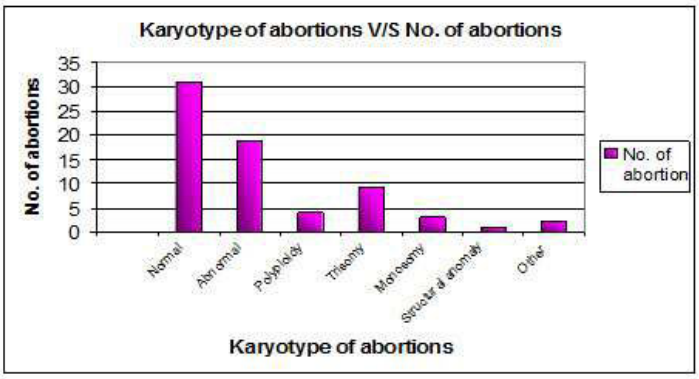
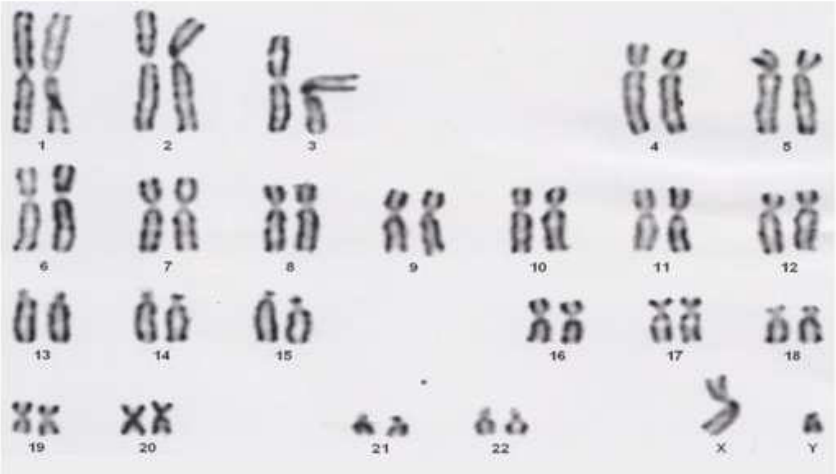
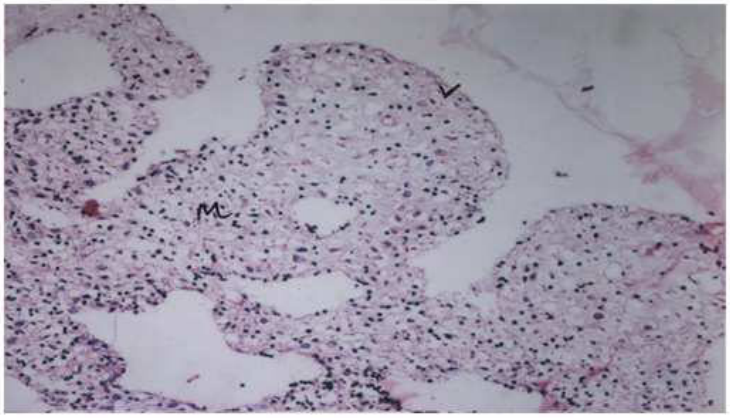


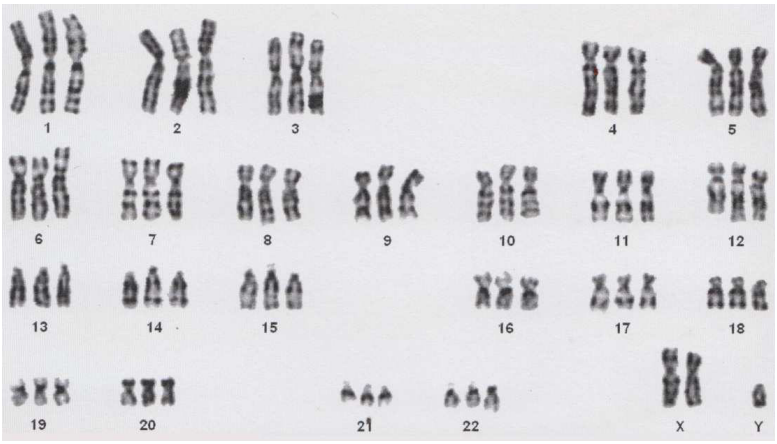

**Table2**
***Summarizes the relation of number of cases with karyotype of abortion. The frequency of chromosomal abnormal abortion due to trisomy was found to be maximum that is (47.4%) followed by polyploidy (21.1%), monosomy X (15.8%), structural anomalies (5.2%) and other abnormalities (10.5%).***



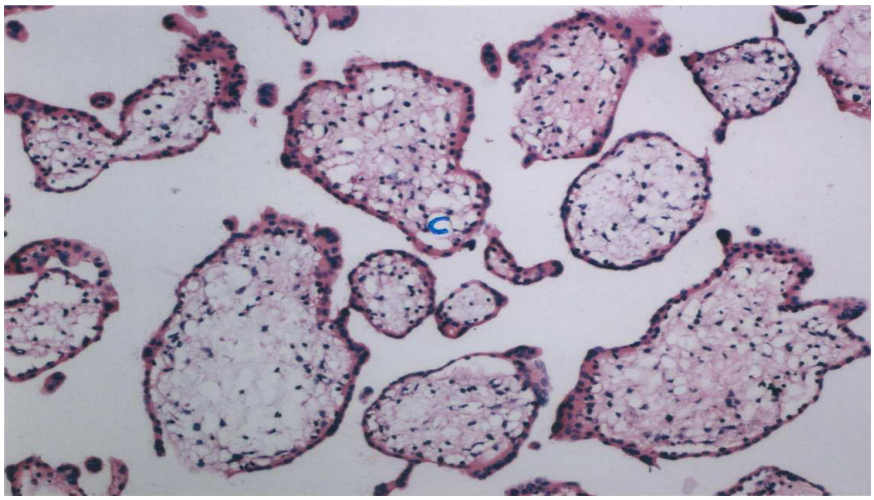
***CASE -9 Karyotype - 46XY (G- Banding)***



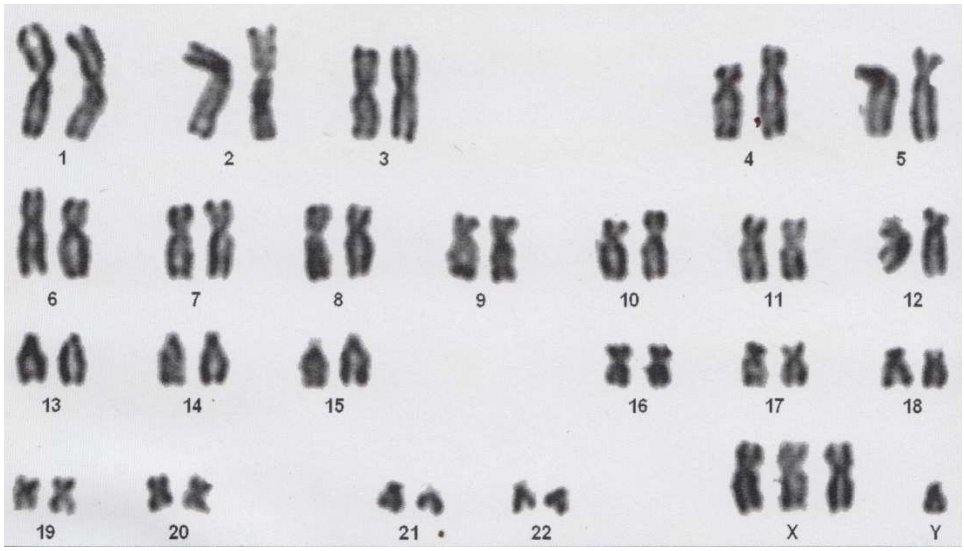

***CASE -9 - 46XY,H and E stain, Low Magnification***
***V- Villus M-Mesenchymal Core***



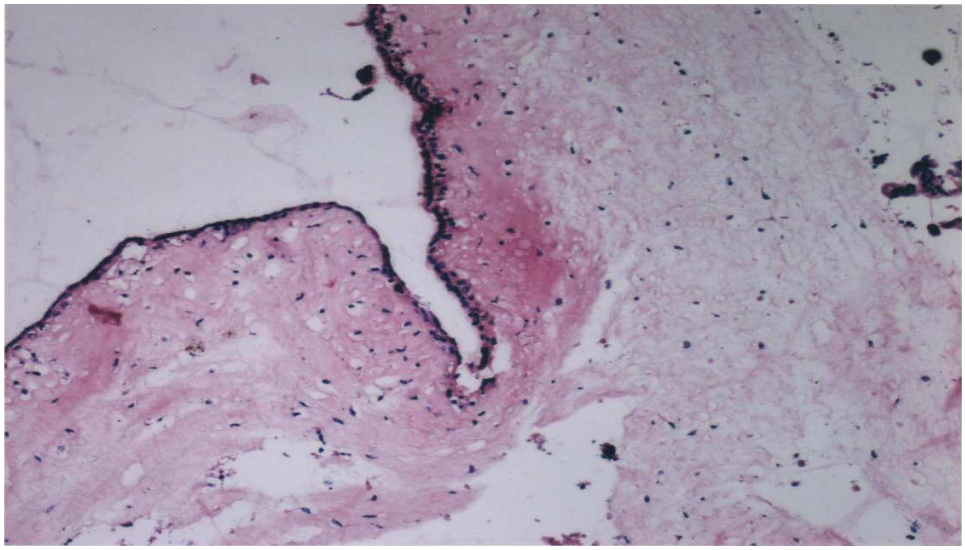

*CASE-11 Karyotype - 69XXY(Triploidy)(G- Banding)*




*CASE -11Triploidy, H and E Stain, Low Magnification*
*V-Villus C-Cystic Changes in Mesenchyme*




*CASE-15 Karyotype 48XXXY (Polysomy)(G- Banding)*



*CASE -15 Polysomy – H and E Stain, Low Magnification*

## RESULTS

Histology and cytogenetic analysis from 50 samples of products of conception received after 1st trimester abortion was done. The maternal age of the mother ranged from 19 to 40 years. Abnormal karyotype were found in 38% of the cases investigated. Two of them showed double cell line. The frequency of chromosome anomalies was highest in abortions of 8-9 weeks of gestation (Table 1). Trisomy was predominated (47.4%), followed by polyploidy (21.1%), Monosomy X (15.8%), structural anomaly (5.2%) and other abnormality was (10.5%). One structural abnormal karyotype showed translocation 46, XY t (13q:14q). Two triploid abortions had XXY, one had 47, XX + marker (65%), 70,XXX+marker (35%), and one had 48,XXXY sex chromosome constitutions (Table 2). The number of abortions were maximum in 8-11 weeks of gestations.

### *HISTOLOGY STUDY*

Triploidy shows an increase in the villus size with irregular outline of villi. Focal trophoblastic hyperplasia is seen. Vacuoles are formed in the mesenchymal core. Hypovascularity of the villus is seen. Monosomy X- shows small hypovascular villi with hypoplastic trophoblast. Polysomy- shows increase in villus size with irregular outline of villi. Trophoblastic hyperplasia is seen. Hypovascularity of the villus is observed. Moderate amount of infiltration is seen. Trisomy- irregular outline of the villi with hypovascularity was observed.

## DISCUSSION

Spontaneous abortion provide an excellent source of material for investigation into the nature of human chromosome abnormality[7]. About 90% of spontaneous abortion occur in the first trimester of pregnancy[8]. The high frequency of pregnancy wastage is predominantly (40-50% of all cases) caused by chromosomal anomaly[9]. Knowledge of the karyotype from an abortion is clinically relevant for prognostic and therapeutic reasons[9]. It may be important to investigate the karyotypic status of abortion in view of potentially serious consequences for future pregnancy[10]. Chromosomally abnormal pregnancies are seen to be associated with histological changes of chorionic villi and of the placenta[9]. There have been numerous attempts to correlate specific chromosome anomalies with the histology of placenta[8]. The only way that the incidence, origin and aetiology of such chromosome abnormality can be studied by the examination of spontaneous abortion[7]. In the present study individual samples of chorionic villi were divided and subjected for

cytogenetic analysis and histological study. Histological preparation were examined without knowledge of the karyotype to evaluate the precision and significance of placental histology for categorizing chromosomally normal and abnormal products of spontaneous abortion[9]. Cytogenetic analysis of spontaneous abortion provide valuable information on the frequencies of chromosomal anomalies[7]. In our study the rate of abnormality is 38 % of the case investigated. Eiben et al 1987 found the abnormal karyotype to be 48.6% of the cases investigated[11]. Hassold et al 1980 reported that approximately 50% of the samples were chromosomaly abnormal[7]. The overall rate of abnormality were in close agreement with data from other Cytogenetic study of spontaneous abortion Lauristen (1976)[12]. Rehder et al 1989 showed the overall abnormality to be 58.5% of the cases[8]. M. Geisler et al 1978 study the rate of chromosomal aberration to be 39%[13]. Minguillon et al 1989 showed the overall rate of chromosomal anomaly is 49.6%[9].

In our study the chromosomal abnormalities are highest in 8-9 weeks of gestation. Eiben et al 1987 reported the chromosomal anomalies to be highest in 10-11 weeks of gestation[11]. Hassold et al 1980 reported the percentage of abnormal chromosome complement to be highest between 12-15 weeks of gestation[7]. Ohno et al 1991 reported the rate of chromosome abnormality to be maximum in 8-9 weeks (70.5%)[14]. In our study the chromosomal analysis showed trisomy as the predominant abnormality 47.4%, followed by polyploidy21.1%, monosomy X 15.8%, structural abnormalities 5.2% and other 10.5 %. Eiben et al 1987 reported that in the chromosomal anomalies trisomy was 66.2% followed by polyploidy (22.1%) and monosomy X (7.4%) and structural abnormalities (4.4%)[11]. M. Geisler and J.Kleinbrecht et al 1978 reported trisomy to be 60% followed by monosomy X (20%), triploidies (14%) and structural aberration (6%)[13]. Hassold et al 1980 pointed out Trisomy to be the pre-dominant abnormality (44.5%) followed by monosomyX (24.2%), triploidy (15%), tetraploidy (7.1%) followed by structural abnormality (4.3%)[7]. The overall rate of abnormality and the proportion of different types of abnormality was in close agreement with data from other cytogenetic studies of spontaneous abortion Lauristen 1976[12].E.Jauniaux et al 1992 showed trisomy to be the most pre-dominant abnormality (36.9%), monosomyX (25.4%), triploidies (25.4%) and other chromosomal rearrangement (12.3%)[15]. Ohno et al 1991 showed that the distribution of abnormal chromosome complement with Trisomy to be 64% followed by polyploidy 9%, monosomyX 7%, structural rearrangement 6%[14].

### *HISTOLOGY PART*

Chromosomally abnormal pregnancy seen to be associated with histological changes of chorionic villi and of placenta. The predictive value of placental histology for categorization of the products of chromosomal normal and abnormal spontaneous abortion has been considered to be fairly accurate to date[9]. There have been numerous attempts to correlate specific chromosome anomalies with the histology of placenta. It has been shown that triploid may lead to extensive hydropic changes of the villi resulting in partial hydatiform mole. Trisomies 16 and 20 causes developmental arrest and less severe hydrops of villi. Trisomies 13,18,21 shows less marked disturbance of placental maturation and villous edema. Monosomy X shows focal hydropic changes and focal fibrosis of placenta[8]. In our study the highest precision in categorizing chromosomal abnormal abortion by histology reached with triploidy, polysomy. Triploidy shows increase in the villus size with irregular outline of villi. Focal trophoblastic hyperplasia is seen. Vacuoles are seen in the mesenchymal core. Hypovascularity of the villus is seen. Monosomy X shows small hypovascular villi with hypoplastic trophoblast. Polysomy shows increase in villus size with irregular outline of villi. Trophoblastic hyperplasia is seen. Hypovascularity of the villus is seen. Moderate amount of infiltration is present. Trisomy shows irregular outline with hypovascular medium sized villi. Based on the idea that abnormal genetic make up results in abnormal development and structure of chorionic villi and concept that each genetic abnormality is characterized by specific structural deviation, many studies have been undertaken to explore the relationship between

karyotype and histological feature in the early placentas. Honore et al (1976) have evaluated placental histology from 112 such abortuses with abnormal karyotype and 36 abortuses with normal karyotypes. They concluded that accuracy of a karyotypic diagnosis made solely on the basis of placental histology is in the range of 80 %. He suggested that in spontaneous abortion diffuse villous hydrops with prominent individual intravillous cytotrophoblast cells is associated with autosomal trisomies, hypoplastic, hypovascular fibrotic vill are associated with monosomy X and hydropic villi, villous haemorrhage and infarction are associated with tetraploidy[8].E. Jauniaux and J. hustin et al 1992 showed the coorelation between cytogenetic and histological examination and demonstrated with specificity of 65.5 % and sensitivity of 45 %[15].Novak et al 1988 and minguillon et al 1989 reported correlation of 59 % and 55% between cyotgenetic and histology. Both author concluded that placental villous morpholgy is an insensitive indicator of chromosomal anomalies[16]. Rehder et al 1989 and rocklein et al 1990 using both patho- morphological classification and morphometric analysis were able to increase the predictive value of histology for identifying chromosomal aberrations[8].

In our study significant coorelation was found in cases of spontaneous abortion presenting with triploidy. MonosomyX is not associated with obvious villous morphological anomalies and escape microscopic recognition. Trisomy is not associated with prominent villous alteration.J. Hustin, Jauniaux et al 1992 in their study of 184 complete spontaneous abortion found reduced trophoblastic penetration into the decidua. Trophoblastic columns are reduced in number and trophoblastic shell is thinner and discontinues in case of spontaneous abortion with an abnormal karyotype[15]. Minguillon et al 1980 found a 22.5 % incidence of abnormal villous histological findings in case of spontaneous abortion with normal karyotype[9]. Novak et al 1988 were the first to study individual histological features 75 placentae were assessed for the presence of hydropic changes, fibrohyalinization, irregular shape with pseudoinclusion, cytotrophoblastic cells in the stromal core and lymphocytic aggregation with signs of acute inflammation of the decidua. Only irregular shape of chorionic villi with pseudoinclusion appeared to be of relevant for recognition of abnormal karyotype[16].

## CONCLUSION

Histology study and cytogenetic analysis from 50 samples of products of conception was done. The frequency and type of chromosome anomalies were detected. Abnormal karyotype were found in 38% of the cases investigated. Trisomy predominated (47.4%) followed by polyploidy (21.1%) monosomyX (15.8%), structural anomaly (5.2%) and other abnormalities (10.5%). Thus in conclusion from above study cytogenetic analysis is necessary for differentiating between chromosomally normal and abnormal abortion for further management of the patient. Regarding histology study the highest precision in categorizing chromosomal abnormal abortion reached with triploidy and trisomy. Monosomy X was not associated with obvious villous morphological change. Thus it was seen that placental microscopic examination alone was not enough to give a proper aetiological diagnosis in cases of spontaneous abortion. Thus chorionic villus histology helps to differentiate between chromosomally normal and abnormal abortion.

### CONFLICT OF INTEREST

Conflict of interest declared none.

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