# Comprehensive post-mortem evaluation in Intrauterine deaths /still births in improving uptake of autopsy and future pregnancy outcomes

Seema Thakur[1], Khushboo Kadam[2], Preeti Paliwal[2], Shubhnita Singh[1], Chanchal Singh[3], Rachna Gupta[4], Savita Dagar[5], Sudhir Jain[2], Akshatha Sharma [6], Renu Saxena[2], Kamal Saxena [2], Sudha Kohli[2], Vandana Thareja[2], Rajni Saxena [2], Renu Caprihan[2], Vimal Grover[2], Manorma Bhutani[2], Anita Gupta [2], Neena Kumar[2], Vandana Gupta [2], Mamta Mittal [2], Sunita Verma[2], Arpana Jain[2], Jyoti Chakerverty [2], Mukta Kapila[2], Swati Mittal [2], Sunita Kapoor[2], and Deepa Khurana[2]

[1]Fortis Hospital Shalimar Bagh
[2]Affiliation not available
[3]Birthright, by Rainbow Hospitals, New Delhi
[4]Sunehri Devi Hospital
[5]Birthright, by Rainbow Children's Hospitals, New Delhi
[6]Indraprastha Apollo Hospital

March 07, 2024

**Abstract**

Objective: Post-mortem examination of a baby following spontaneous or missed miscarriage in the second trimester or intrauterine death to provide a complete or partial explanation of the pregnancy loss. Method: A total of 100 cases of intrauterine deaths were consecutively collected from January 2010-May 2019 for etiological diagnosis according to the standard protocol and involved external examination, dysmorphological examination, internal examination and full body antero-posterior and lateral radiographs. Histopathology of placenta was done. Cases were also subjected to genetic testing such as FISH/microarray. A clinical correlation was done by a Obstetrician-geneticist to reach an etiological diagnosis. Results: Two third of cases were referred after intrauterine death post 30 weeks of gestation. 24/100 cases were with fetal anomalies.Genetic causes present in 12% cases. 65.5% cases were associated with the pathology of the placenta. 30% cases were with cord lesions. The results were inconclusive in 14% of the cases. Conclusions: In this study we looked for establishing etiological diagnosis and tried to see contribution of each test in finding the cause. This will help the obstetrician in counselling parents for the utility of post-mortem excamination and thus better able to guide for future recurrence risk and management.

**Comprehensive post-mortem evaluation in Intrauterine deaths /still births**

**in improving uptake of autopsy and future pregnancy outcomes**

Dr Seema Thakur[1], Dr. Khushboo Kadam[2], Dr.Preeti Paliwal[3], Dr.Shubhnita Singh[4], Dr Chanchal Singh[5], Dr. Rachna Gupta[6], Dr. Savita Dagar[7], Dr. Sudhir Jain[8], Dr. Akshatha Sharma[9], Dr Renu Saxena[10], Dr. K.K Saxena[11], Dr.Sudha Kohli[12], Dr Vandana Thareja[13], Dr Rajni Saxena[14], Dr.Renu Caprihan[15], Dr. Vimal Grover[16], Dr. M Bhutani[17], Dr. Anita Gupta[18], Dr. Neena Singh Kumar[19], Dr. Vandana Gupta[20], Dr. Mamta Mittal[21], Dr. Arpana Jain[22],Dr. Sunita Verma[23], Dr Jyoti Chakroborty[24], Dr. Mukta Kapila[25], Dr. Swati Mittal[26], Dr Sunita Kapoor[27], Dr. Deepa Khurana[28]

1,2,4- Dept. Of Genetic and Fetal Diagnosis, Fortis hospital Delhi-NCR

3,10,12- Institute of Medical Genetics and Genomics, Sir Ganga Ram Hospital, New Delhi

5-Senior Consultant, Dept. of Fetal Medicine, Rainbow Children Hospital, Delhi

6- Senior Consultant, Dept. of Fetal Medicine Sunhari Devi Hospital, Sonepat

7. Senior Consultant, Dept. of Fetal Medicine, Venkateshwar Hospital, Delhi

8- Emeritus Professor, Deptt of Pathology

9- Senior Consultant, Dept. of Fetal Medicine, Apollo Hospital, Delhi

11. Senior Consultant, Dept. of Radiodiagnosis, Sir Ganga Ram Hospital, Delhi

13- Consultant Radiologist, Vandana Diagnostic, Malviya Nagar, New Delhi

14, 15- Senior Consultant, Dept. of Radiodiagnosis, Fortis Lafemme, Delhi

16-19- Senior Consultant, Dept. of Obs & Gynae, Fortis Lafemme, Delhi

20-23- Senior Consultant, Dept. of Obs & Gynaecology, Fortis Hospital, Shalimar Bagh, New Delhi

24- Senior Consultant, Dept. of Pathology, Fortis Hospital, Shalimar Bagh, New Delhi

25,26- Senior Consultant, Dept. of Obs & Gynaecology, Fortis Memorial Research Institute, Gurgaon

27,28- Senior Consultant, Dept. of Pathology, City X- ray, Tilak Nagar, New Delhi

**Running title:** Post- mortem evaluation in still births/IUD

**Corresponding Author:**

Dr. Seema Thakur

Fortis Hopital, AA Block, Shalimar Bagh, New Delhi

Fortis lafemme, GK 11, New Delhi

Email: seematranjan@gmail.com

**Tweetable abstract:**

Post-mortem examination of a baby following spontaneous or missed miscarriage in the second trimester or intrauterine death may provide a complete or partial explanation of the pregnancy loss. Autopsy is the single most useful investigation and provides information that adds to the clinical diagnosis in nearly half of cases and is also a valuable audit of clinical care.

We have analysed 100 cases for etiological diagnosis in still birth/ intrauterine deaths and suggest that post mortem comprehensive evaluation has a diagnostic yield of 86% and reducing the unexplained causes to 14%

**ABSTRACT**

**Objective:** Post-mortem examination of a baby following spontaneous or missed miscarriage in the second trimester or intrauterine death to provide a complete or partial explanation of the pregnancy loss.

**Method:** A total of 100 cases of intrauterine deaths were consecutively collected from January 2010-May 2019 for etiological diagnosis according to the standard protocol and involved external examination, dysmorphological examination, internal examination and full body antero-posterior and lateral radiographs. Histopathology of placenta was done. Cases were also subjected to genetic testing such as FISH/microarray. A clinical correlation was done by a Obstetrician-geneticist to reach an etiological diagnosis.

**Results:** Two third of cases were referred after intrauterine death post 30 weeks of gestation. 24/100 cases were with fetal anomalies. Genetic causes present in 12% cases. 65.5% cases were associated with the

pathology of the placenta. 30% cases were with cord lesions. The results were inconclusive in 14% of the cases.

**Conclusions** : In this study we looked for establishing etiological diagnosis and tried to see contribution of each test in finding the cause. This will help the obstetrician in counselling parents for the utility of post-mortem excamination and thus better able to guide for future recurrence risk and management.

**Key words**

Intrauterine death, still birth, Fetal Autopsy, chromosomal microarray, placental histopath, short cord, genetic counselling

**Introduction**

Fetal death is defined as the delivery of a fetus showing no signs of life as indicated by the absence of breathing, heart beats, pulsation of the umbilical cord, or definite movements of voluntary muscles.[1]It is divided in early (<22 weeks of gestation), intermediate (between 22 and 27 weeks of gestation) and late (> 28 weeks of gestation) depending on gestational age. Of these, early are designated as abortions whereas intermediate and late are known as stillbirths.[1]

Intrauterine fetal death (IUFD) at any time during pregnancy is devastating for the expecting couple as well as their families and it is at such times that issues like the cause of the death of the fetus and the fear of recurrence of such mishap in future pregnancies crop up. These are major concerns for the couples as well as care givers; hence the determination of cause and mechanism of death is important to facilitate counseling of parents, management of subsequent pregnancies and future interventions if possible. However majority of times these questions remains unanswered due to several underlying factor and hence counseling parents is often unsatisfactory.

Despite these limitations of ascertaining the precise cause it is important to identify etiological factors that might themselves contribute in IUD or can be indicative of other factors that can play a significant role in IUD. The causes include maternal, fetal, placental lesions, cord accidents and genetic causes that should be investigated as per ACOG guidelines as a part of postmortem investigation of intrauterine death.[2]In the present study we have analysed 100 cases of IUFD. Our primary objective was to calculate the importance of various investigations in systematic postmortem examination protocol. This will help the obstetrician in counselling parents for the utility of post-mortem excamination and thus better able to guide for future recurrence risk. Such protocol based evaluations are much needed in our country as India is listed as a country with highest number of still birth.

**Material & Methods:**

We included 100 fetus in this series which were referred for fetal post-mortem for intrauterine deaths during January 2010- May 2019.we included all cases more than 12 weeks of gestation. These were referred from various hospitals and clinics across Delhi-NCR to us. All the cases included in the study had no anomaly detected on the ultrasound. Our centre is involved in fetal autopsy and is a referral centre for such work. Parental consent was taken in all cases after counselling about the utility of fetal autopsy. Fetal post-mortem involved external examination, dysmorphological examination by the clinical geneticist, internal examinations, full body antero- posterior and lateral radiographs. Histopathology studies were advised for fetal organs and placenta in all cases and genetic testing was done in all cases that included karyotyping and in case of culture failure reflex FISH/QF PCR studies were done for chromosomes 13, 16,18,21, 22 and sex chromosomes before 2016. After 2016, microarray (mainly 315 k) was the preferred test. Additionally DNA storage was done for all cases for further genetic evaluations if needed. A final interpretation and correlation was done by an obstetrician- clinical geneticist to correlate all fetal investigations to identify underlying etiology.

We have complied with the ethical conduct of research involving human subjects as per Helsinki declaration. Consent for autopsy and all investigations were taken from all cases in the study. Consent for including in

research was also obtained in every case.

Results:

From January 2010 to May 2019 a total of 100 intrauterine fetal deaths were included in the study.

Median age of mothers was 30.63 years (22–39 years) and median gestational age at determination of IUD was 24 weeks and 4 days (12 weeks and 0 days– 40 weeks and 0 days) (Table 1). Number of male fetuses was 54; whereas female fetuses were 43 and remaining three had ambiguous genitalia. 98 cases involved singleton pregnancy and only 2 involved twin pregnancies. Growth restriction was present in 48% cases. Two cases had overgrowth.

Distribution of etiological diagnosis is depicted in Table 2.

Autopsy showed external anomaly in 16 out of 100 cases whereas internal anomaly was noted in 8 cases (Table 2). External anomaly involved mainly ear malformations and digit amputations and these were not picked up on ultrasound. (Fig 1) Of the 8 cases with internal anomaly three had malformations of gastrointestinal tract including volvulus (Table 3). Infantogram suggested skeletal dysplasia in one case with short limbs.

Short cord ($<10^{th}$ centile for gestation) was present in 22 cases. (Table-3). One case has long cord. Nuchal cord was present in 2 cases (Table 3, Fig 2). 4 cases had associated single umbilical artery. Hypocoiling was observed in cases with short cord whereas hypercoiling was also noted (Fig-3 a,b,c). Placental histopathology was done in 83% of cases. (Table 2) Placental lesions were present in 65.5% of cases (Table-4). Placental infarction was seen in 14 cases (Table 3,Fig-3d & e). Placental haemorrhage was found in 14 cases (Table 3, Fig-4). In 12 cases placental examination showed chorioamnionitis (Table-3)

Genetic analysis was performed in 93% cases with intrauterine death (Table 2). Before 2016, fluorescence in-situ hybridization (FISH) for 5 common aneuploidies was performed for 40 out of 57 cases (FISH was done after culture failure). After 2016, Single Nucleotide Polymorphism (SNP) chromosomal microarray was offered in 36 cases. 12% cases were of genetic disorders and this included 8 cases of common aneuploidy of chromosome 21, 18, 16 and sex chromosome aneuploidy, one case of abnormal microarray three syndromic cases- two of Treacher Collin syndrome and one case of suspected Beckwith Weidmann syndrome (Table 3, Figure 4). We found 2 cases of trisomy 21 and 2 cases of 47, XYY. We obtained one case each of trisomy 18, trisomy 16, monosomy 21 and triploidy. Microarray showed 19p deletion in one case (Table 3)

The eliological detection rate for IUD/stillbirth are represented in Table-4

Figure 5 shows venn diagram of all investigations done and the association of multiple etiological factors in this study.

**Discussion:**

Our study highlights the importance of comprehensive analysis for all stillbirth/IUD cases. This study has important implications for clinical practice and research. In our study we identified that cord (30%) and placental abnormalities (65.5%) are associated with major causes of still birth (Table 2). We further segregated these placental changes into subcategories and of which infarction and haemorrhage were major contributors (Table 3;fig 3 d,e, 4). We found genetic causes in 12% cases and the cause of death was unexplained in 14% cases.

Kortwig and colleagues present data on the analysis of 1025 cases of still births.[3]In this study placental examination was found helpful in 95.7% of cases, autopsy was helpful in 72.6% cases, followed by cytogenetic analysis in 20% cases. Secondary analysis of 512 cases of still birth was done by Page etal in still birth collaborative research 2006-2008.[4] Usefulness of placenta histopathology was 64.6%, fetal autopsy 42.4% and genetic analysis 11.9%. We noted placental lesions in 65.5% cases. Man et al conducted a study on 1064 intrauterine deaths and noted that in around 30-60% % of cases, the cause of death was unexplained even after careful clinical review, fetal external examination/imaging and placental examination.[5]

Our data however showed a higher percentage of associated pathology which can be explained by systematic analysis of cases in a dedicated unit. Pekkola et al did a standardized postmortem examination and a re-evaluation of the results and report unexplained cases in only 10.2% cases.[6]

Autopsy that included thorough evaluation of fetus revealed fetal growth restriction in 48 cases. With the advancement of technology and follow up ultrasonography, majority of fetal malformations can be identified in utero, however external malformations are very difficult to identify and can be easily seen on external examination of fetus. These not only help in the characterisation of the fetal abnormalities but can further help in delineating the course of further investigations or syndromic diagnosis.

In the present study genetic testing was undertaken for ninety three percent cases and majority of cases were subjected to karyotype and in case of culture failure FISH test was undertaken.

Microarray was introduced at a later stage as first tier test for product of conception. We could identify a genetic cause in 12% percent of cases. This percentage can be higher if microarray is done for all cases of still births. (Table 2) Previous studies have shown that chromosomal abnormalities are present in 6–13% of stillbirths and this percentage is higher in pregnancies with fetal malformations.[7]The difference in the percentages can be partly due to the cell culture failure and partly because of limited resolution of karyotyping. Unfortunately, cost is a barrier for many patients and it may not be feasible to perform in all cases.

In our study we were able to identify two cases of Treacher Collins syndrome and one case of Beckwith Weidman syndrome based on the phenotype. This stresses on the fact that extensive analysis should be undertaken before samples are sent for genetic analysis and deciding the most cost effective test. Gross dysmorphism is indicative of underlying chromosomal abnormalities or genetic syndrome. Our study with these examples is thus suggestive of a need for comprehensive analysis that can be informative and can be cost effective.

It has been noted that most of the abnormalities are not seen in isolation and thus a combined risk assessment is much needed. In our study we identified an overlapping in cause in 20 % with maximum number of overlaps seen in fetal growth restriction and placental abnormalities. Altered fetal growth and placental abnormalities are the strongest and most prevalent known risk factors for stillbirth. However, most pregnancies with placental abnormalities or fetal growth aberrations do not result in stillbirth.

Counseling for utility of fetal autopsy and genetic testing is important to improve the uptake of autopsy. Due to lack of knowledge in this area, health care personnels are unable to convince the parents about benefits of autopsy and this leads to decreased uptake. Kortwig et al suggest that basic fetal workup of still birth should include three tests: autopsy, placenta histopathology and cytogenetic investigations. [3]. They suggest this approach to be most cost effective. ACOG practice guidelines also suggest that most important investigation in such evaluations are autopsy, placenta histopathology and karyotyping.[2] However, perinatal autopsy is underutilised in most of the centers. There are multiple factors with regard to this but parental refusal to autopsy seems most important reason. This refusal is mainly due to the inability of the treating obstetrician to communicate regarding the utility of fetal autopsy. The present articles thus adds the following points that clinicians/ counsellors can highlight regarding the detection rates while counselling to increase the uptake of fetal autopsy and genomic investigations (Table 4)

1. Clinical evaluation by an experienced clinical geneticist can provide an answer to 16%.
2. Internal post mortem adds to further 8%
3. Genetic tests are abnormal in 12% cases.
4. Placental histopathology is helpful in 65.5% cases
5. Cord abnormalities are noted in 30% cases
6. Complete evaluation can identify cause 86% of such cases

**Conclusions:** Counselling for utility of fetal autopsy and genetic testing is important to improve the uptake of autopsy. The present manuscript thus adds the following points that counsellors can highlight regarding the detection rates while counselling such as: clinical evaluation by an experienced clinical geneticist, internal post-mortem; and placental histopathology and genetic analysis to detect possible abnormalities. This will help in the informed decision making by the parents and the cost effective analysis for etiological diagnosis of these cases and thus improving the standards of care in prevention and further guide research in this complex and sensitive issue. An understanding of these interrelationships may contribute to a better understanding of stillbirth mechanisms and thus a prenatal identification of placental findings and fetal growth abnormalities can improve stillbirth prediction and thus prevention.

**Acknowledgements**

Anisha Raju, THB , Gurugram for technical help in formatting article, fig and tables

**Conflicts of Interests: NoneFunding- NoneEthics statement-**

Ethical clearance: This was a retrospective study so ethical clearance was not taken

We have complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects

**Contribution to Authorship**ST(1)- Main person doing the entire study KK(2)- Assistant to ST SS(4)- Assistant to ST PP(3)- Cytogenetecist, Helped in manuscript writing and data collection RS(10),SK (12)- Molecular Geneticist doing genetic testing CS(5)- Fetal medicine consultant involved in care of the patients and data collection RG(6)- Fetal medicine consultant involved in care of the patients and data collection SD(7)- Fetal medicine consultant involved in care of the patients and data collection SJ(8)-Pathologist AS(9)- )- Fetal medicine consultant involved in care of the patients and data collection VT(13)- Radiologist for infantograms KKS(11)- Radiologist for infantograms RS(14),RC(15)-Radilogist involved in patient care MB (16),VG(17), AG(18),NS(19)- Obstetrician involved in care of the patients and data collection VG(20), MM (21)AJ (22),SV(23)- Obstetrician involved in care of the patients and data collection JC(24)- Pathologist involved in patient care MK(25),SM(26) –Obstetrician involved in care of the patients and data collection SK(27),DK (28)- Pathologist involved in patient care

**References**

1. National Center for Health Statistics. State definitions and reporting requirements for live births, fetal deaths, and induced terminations of pregnancy. 1997 revision. Hyattsville (MD): NCHS; 1997. Available at: http://www. cdc.gov/nchs/data/misc/itop97.pdf. Retrieved November 19, 2008. (Level II-3)
2. Ruth C. Fretts, Clinical management guidelines for obstetrician–gynaecologists. ACOG practice bulletin. Number 102. 2009. 113, 748-761.
3. Korteweg FJ, Erwich JJHM, Timmer A, et al. Evaluation of 1025 fetal deaths: proposed diagnostic workup. Am J Obstet Gynecol 2012;206: 53.e1-12.
4. Page JM, Lindquist L C, Thorsten V,Parker C B, Reddy U M, Dudley D J Diagnostic Tests for Evaluation of Stillbirth Results From the Stillbirth Collaborative Research Network , Obstet Gynecol 2017;129:699–706
5. Man J, Hutchinson J C, Heazell A E, M. Ashworth M, Levine S and N. J. Sebire N J. Stillbirth and intrauterine fetal death: factors affecting determination of cause of death at autopsy .Ultrasound Obstet Gynecol 2016; 48: 574–578
6. Pekkola M, Tikkanen M , Loukovaara M, Lohi J, Paavonen J and Stefanovic. Postmortem examination protocol and systematic re-evaluation reduce the proportion of unexplained stillbirths. J Perinat Med 2020;48(8)771-777
7. Reddy UM, Page GP, Saade GR. Karyotype versus microarray testing for genetic abnormalities after stillbirth. N Engl J Med. 2012 Dec 6;367(23):2185-93.

**Legends to Figures**

Fig-1: IUD at 35 weeks in Suspected Treacher Collin syndrome evere Retrognathia, absent mandible, hypoplastic maxilla, High arched palate, low set ears bilateral, Left ear- absent upper part of ear, absent external auditory meatus on left side, Right ear: Accessory ear tag, narrow external auditory meatus

Fig-2: IUD at 34 weeks, Nuchal cord, Hypocoiling

Fig-3: (clockwise)Placental and cord lesions associated with IUD

3a-Hypocoiling, Abnormal decrease in the number of coils of umbilical cord.

3b-Marginal cord insertion, Umbilical cord is attached close to disc margin.

3c-Hypercoiling, Increased coils > 2 coils per 5 cm, can cause significant fetal retardation and death

3d-Perivillous fibrin deposition, Thickened placenta with pale grey areas of fibrin deposition

3e-Infarction, A pale grey area of late infarct

Fig-4: RetroplacentalHaemorrhage, Dark red hemorrhagic clotson maternal surface.

Fig-5: Venn diagram showing the overall distribution of cases and the overlapping spectrum of different underlying abnormalities

**Table-1 Case Summary**

| **Categories** | **Number (N)** |
|---|---|
| **Age of Mother (Years)** | **Age of Mother (Years)** |
| 20-24 | 8 |
| 25-29 | 43 |
| 30-34 | 39 |
| 35-40 | 9 |
| **Gestation age at IUD/Still birth (Weeks)** | **Gestation age at IUD/Still birth (Weeks)** |
| 13-20 | 37 |
| 21-27 | 36 |
| 28-36 | 21 |
| 36-40 | 6 |

**Table-2 Distribution of cases in various categories and cause for IUD**

| **Categories** | **Categories** | **Number** | **Number** |
|---|---|---|---|
| Genetic | Genetic | 12/93 | 12/93 |
| Malformations | External | 16/100 8 | 16/100 8 |
| | Internal | | |
| Placental | Placental | Placental | 57/87 |
| Cord | Cord | Cord | 30/100 |
| Unknown | Unknown | Unknown | 14/100 |

**Table-3- Details of Etiological Diagnosis to determine Intrauterine Death/Stillbirth**

| **Categories** | **Number** |
|---|---|
| **Fetal autopsy findings (n=100)** | **Fetal autopsy findings (n=100)** |
| Dysmorphology | Not conclusive |
| External malformations | 16 |
| Internal malformation | 8 |

| Categories | Number |
|---|---|
| FGR (Fetal Growth restriction) | 48 |
| Overgrowth | 2 |
| **Genetic diagnosis associated with IUD (n=12)** | **Genetic diagnosis associated with IUD (n=12)** |
| Trisomy 21 | 2 |
| Trisomy 16 | 1 |
| XYY | 2 |
| Treacher Collins syndrome | 2 |
| Beckwith Weidman | 1 |
| 19 p deletion | 1 |
| Trisomy 18 | 1 |
| Triploidy | 1 |
| Monosomy 21 | 1 |
| **Placental lesions associated with IUD (n=83)** | **Placental lesions associated with IUD (n=83)** |
| Infarction | 14 |
| Chorioamnionitis | 12 |
| Placental Haemorrhage | 14 |
| Chorioangiosis | 1 |
| Marginal/ Velamentous insertion | 2 |
| Deciduitis | 1 |
| Fibrin deposition | 13 |
| Normal | 22 |
| Not available | 17 |
| **Fetal anomaly associated with IUD (n=8)** | **Fetal anomaly associated with IUD (n=8)** |
| Cardiac anomaly | 1 |
| Volvulus | 2 |
| Urorectal malformation | 1 |
| Hydrops fetalis | 2 |
| Hypoplastic lungs | 1 |
| Skeletal dysplasia | 1 |
| **Cord Lesion associated with IUD (n=30)** | **Cord Lesion associated with IUD (n=30)** |
| Short cord | 22 |
| Long cord | 1 |
| Single umbilical artery | 4 |
| 4 umbillical artery | 1 |
| Nuchal cord | 2 |

**Table-4 Detection Rate of various tests for IUD**

| Test | Detection rate (%) |
|---|---|
| Autopsy external examination | 16 |
| Autopsy internal examination | 8 |
| Genetic testing | 12 |
| Placenta | 65.5 |
| Cord | 30 |

Figure 1 Figure 2

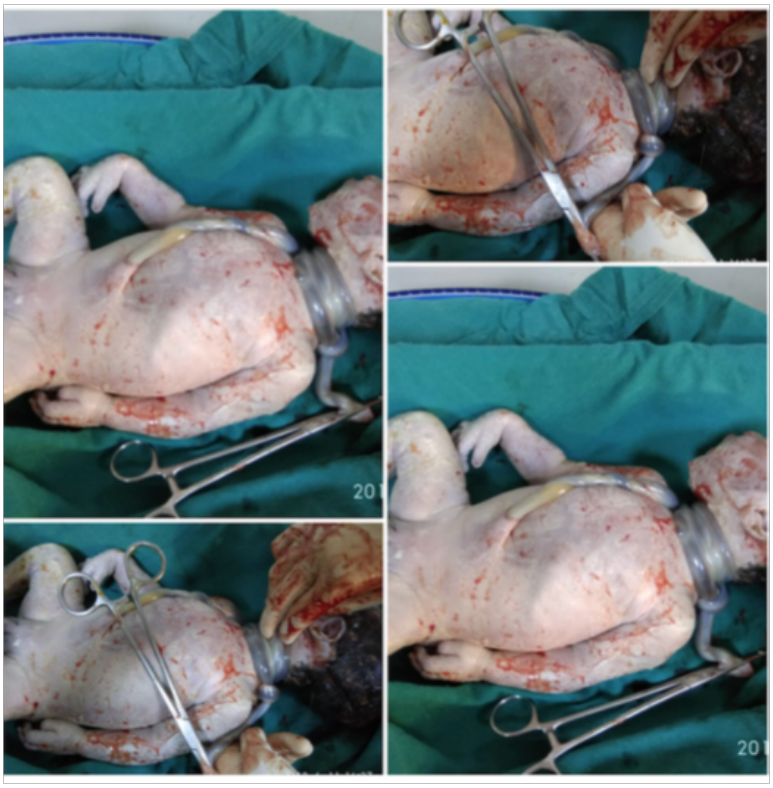

Fig 3

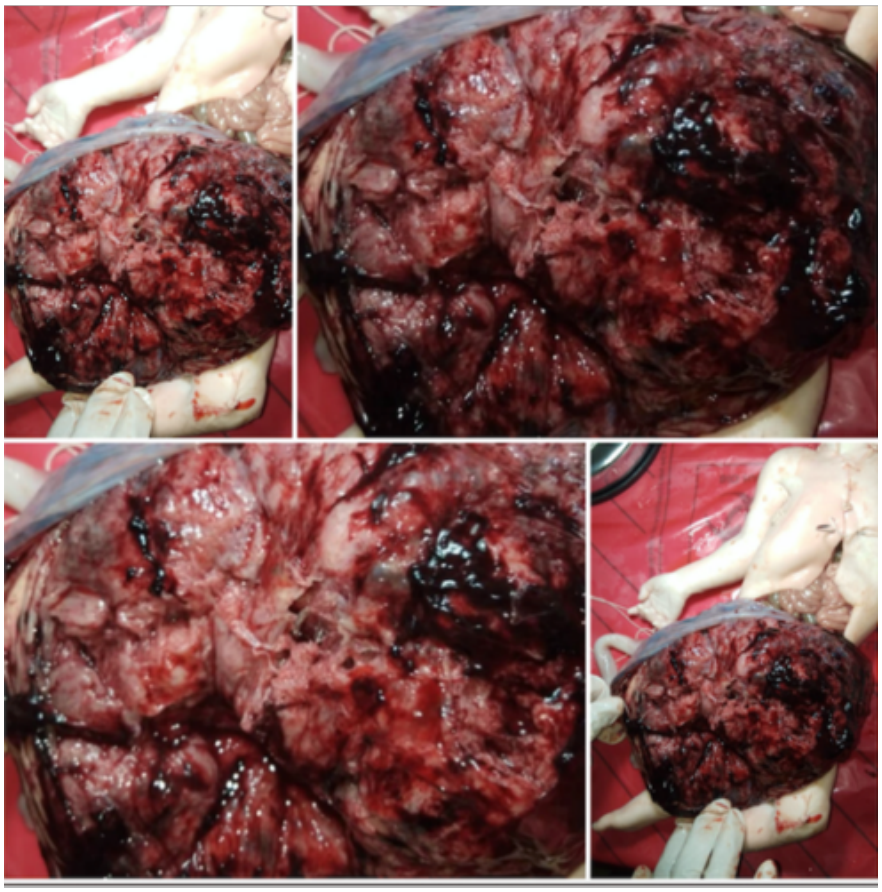

Figure 4

Fig 5