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The pursuit of obstetrics and gynecology over two decades

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Abstract

In the pursuit of fetus as a patient we designed instruments, developed new techniques and evaluated their feasibility, safety and efficacy in patients seeking MTP and in cases referred for prenatal diagnosis in late 80's at AIIMS. Percutaneous ultrasound guided needle aspiration of umbilical cord vessel aided in the diagnosis of various fetal disorders, infections, karyotyping and enzyme deficiency. As it provided access to fetal circulation it was used for intrauterine intravascular blood transfusion for Rh isoimmunized pregnancies and in cases of non-immune hydrops fetalis. A minimally invasive unreported method of percutaneous ultrasound guided fine needle system was used to obtain fetal skin biopsies for diagnosis and management of several devasting skin disorders. Technique of ultrasound guided transabdominal chorion villus sampling was also developed and evaluated. These new techniques provided direct access to the fetus and were of immense value in assessing and managing the unborn fetus.

Uterine balloon therapy was evaluated for the first time in India on hysterectomy specimens, patients undergoing hysterectomy for menorrhagia and those not responding to drugs. Our study indicated that UBT is simple, safe and effective and reduced uterine bleeding in 92.3% of patients with menorrhagia. Invitro tissue culture and sensivity test was carried out to predict response of human ovarian

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cancer and choriocarcinoma cells to select the right anticancer drug on an individual basis. The invitro tissue culture and sensivity test provided the choice of the best drug(s) and the overall prognoses of patients with advanced malignancy could be improved. A new minimally invasive technique for the termination of a pregnancy associated with large and multiple fibroids was also developed. Therapeutic evaluation of Aldactone, Cimetidine and Cyperoterone acetate, and OCP was undertaken for the treatment of hirsutism. Our study on effectiveness of the various drugs in the treatment of hirsutism indicated that hair growth decreased substantially by > 6 points for inpatients treated with Cyperoterone acetate alone or with Aldactone.

Introduction

The fetus *in utero* has been a mystery to humans since time immemorial. Before the introduction of ultrasonography the only method to study the fetus was radiography. Ultrasound opened up a whole new vista of prenatal diagnosis and imaging. But the direct access to the fetus was still lacking.

In 1973 technique of placental aspiration was developed. It was technically simple but it yielded impure samples of fetal blood. However in 1979 Rodeck and Campbell(1) obtained pure fetal blood from the umbilical cord by Fetoscopy. It was a sophisticated technique that required considerable experience to achieve a reliable high standard of performance (2). But it was an invasive technique and was associated with high procedure related complication.

In 1983 Daffos *et al* (3) reported a new technique of percutaneous ultrasound guided needle (20G) aspiration of the umbilical cord vessels that could be performed at any time 17 weeks onwards and could be repeated in the same pregnancy. It has also been used for fetal top – up transfusion (4) as well as for exchange transfusion (5). As it provided access to fetal circulation it was effectively used for intra- uterine intravascular blood and platelet transfusion as fetal therapy in red cell and platelet isoimmunization disorders, respectively (6).

For the diagnosis of several devastating fetal skin disorders, fetal skin biopsy was obtained by the fetoscopic method, which was invasive and was associated with high incidence of fetal loss (7). There were no published reports on ultrasound guided percutanoeus fetal skin biopsy using a finer needle system (8).

Chorionic tissue being representative of the embryo and having a high mitotic index was particularly suitable for rapid cytogentic, enzyme and DNA analysis. In 1982 Kazy *et al* (9) demonstrated the feasibility of transcervical CVS under ultrasound guidance. However, the possibility of implanting infectious agents from the potentially colonized lower genital tract on the growing embryo was considered a major drawback. In 1984 Hahnemann and Smidt – Jensen developed the technique of first trimester transabdominal CVS.

The changing attitudes to abortions, acceptance of small family size, concept of improved quality of life, created a demand for prenatal diagnosis and stimulated scientific endeavor in this field of prenatal diagnosis. Since no work on transabdominal CVS, cordocentesis or fetal skin biopsy was reported from our country, we considered it relevant to evaluate and establish these techniques of prenatal diagnosis and therapy and make the benefits of fetal diagnosis available to all concerned.

Increasing rates of hysterectomies was a matter of great concern; thus the new technology of uterine balloon therapy to treat menorrhagia was also evaluated and used in the Indian setting for the first time.

The use of tissue culture to predict sensitivity of human tumors to various chemotherapeutic agents in the same way as antibiotic sensitivity testing is done with bacteria has long been the dream of oncologists. Thus *in vitro* tissue culture and sensivity test were carried out on ovarian cancer and choriocarcinoma cells to select the right anti cancer drug(s).

Materials and methods

Case selection

Forty patients were selected from the MTP clinic (15 – 20 wks – Experimental group) of All India Institute of Medical Sciences from July1986-89 for ultrasound guided percutaneous fetal umbilical cord blood sampling, fetal skin biopsy and transabdominal chorion villus sampling (first trimester 10-12 wks –25 cases, second trimester 15-20 wks –15 cases). After being satisfied about the feasibility of terminating the pregnancy in accordance with the MTP act of India, a detailed history, thorough physical examination, written and informed consent was obtained from the patients. A preliminary US was performed to note fetal viability, gestational age, number of fetuses, position of placenta, any structural malformation of the fetus, cord, placenta, uterus and cervix. The most convenient and suitable point for the insertion of the sampling needle was noted. FBE, RBS, blood group +Rh type, blood urea, urine routine and microscopic and BT and CT (when indicated) were done.

Fetal Blood Sampling: Technique

Premedication was given with Pethidine (50 mg) and Phenargan (25 mgm) half hour before the procedure. Local anesthesia was given with 1% Xylocaine at the proposed site of puncture over the abdomen. Various sizes needles of 20, 22, 23 and 25 G 20 cm long spinal needles were designed. These needles were sterilized by autoclaving and were flushed with heparinized saline immediately prior to sampling. The long spinal needle (22 G) with stylet was introduced through the proposed site of entry over the abdominal wall. It was gently advanced towards the cord and the umbilical vessel was punctured. The stylet was removed and blood was aspirated. First 0.5 ml of blood was discarded, then 2-3 ml of blood was collected and sent for various investigations. The needle was then removed. Bleeding, if any, from the puncture site on the cord, placenta, and abdomen was noted. The fetal heart rate was recorded during and after the procedure. Instruments and US scan depicting percutaneous fetal cord blood sampling are shown in Fig. 1 and Fig. 2 respectively.

Additional procedure of fetal skin biopsy (15) or transabdominal chorion villus sampling (25+ 15) was done after the fetal blood sampling.

Fetal skin biopsy Instruments and technique

18 G needle 15 cms long with trocar and 25 cms long 20 G cupped biopsy forceps was used. Fetal scalp over the occipital or parietal region was chosen in all cases. By US scanning optimal site was demarcated over the abdomen. Under strict asepsis and local anesthesia over the point of entry 18 G needle with trocar was now introduced under us guidance and the needle tip was slowly advanced till it was 1-2 cms away from the fetal head. The trocar was then removed and free flow of liquor was seen

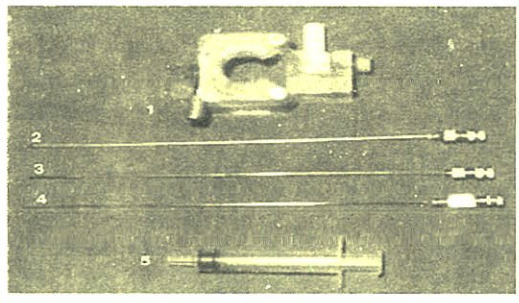

Figure 1
Instruments for ultrasound guided percutaneous fetal cord blood sampling
1. Needle guide, 2,3, 4, needles
2. No. 20 G
3. No. 22 G
4. No 23 G
5. Disposable 2 ml syringe.

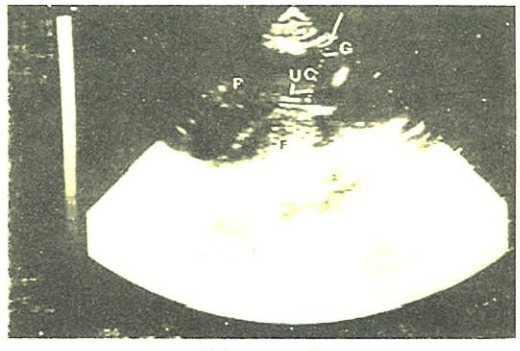

Figure 2

and then 20 G cupped skin biopsy forceps was now introduced through the guide till it protruded through the needle and was slowly advanced till it made the contact with the fetal head. The biopsy forceps was withdrawn little, jaws were opened and then advanced to the fetal scalp and 1 mm of fetal skin sample was obtained. The procedure was repeated and 2-3 samples were obtained. Sometimes due to the fetal movement a second puncture was needed. The total procedure was limited to 10 minutes. After the skin sample had been taken the trocar of the guide needle was reinserted and the needle was removed. Fetal heart rate, bleeding from the puncture site on the skull and abdomen was noted. Instruments used for FSB and US scan depicting the procedure are shown in Figs. 3 and 4 respectively.

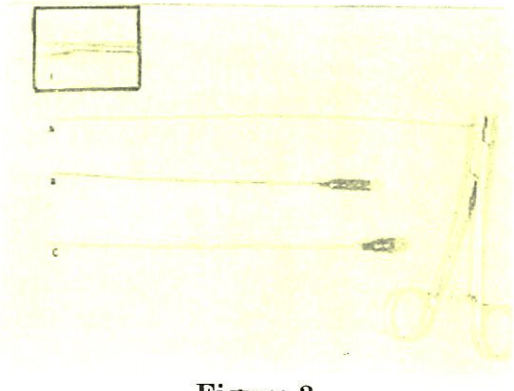

Figure 3
Instruments for ultrasound guided fetal skin biopsy
a. 20 G ,20cm cupped skin biopsy forcep
b. 18G, 18 cm guide needle (cannula)
c. Stylet (Trocar) guide needle
d. Inset magnified view of the cupped biopsy forceps.

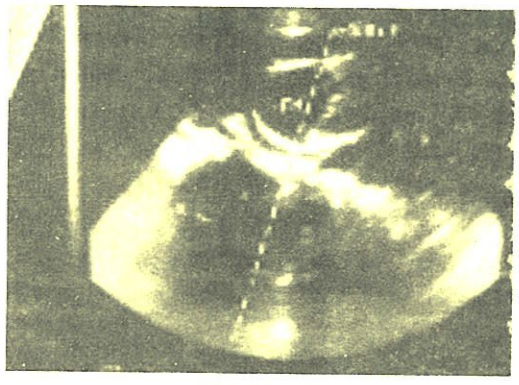

Figure 4

Ultrasound guided transabdominal chorion villous sampling technique and instruments

US scan was done to select the most appropriate site and local anesthesia was given at the proposed site. Under strict asepsis chorion villus sampling was done by the double needle technique of Smidt Jensen (1984). The guide needle was 15 cm long with diameter 18 G. The sampling needle was 20 cm long and had 20 G bore. The 18 G needle was inserted over the proposed abdominal site and it was gently advanced till it reached the placental surface. The stylet was removed. And 20 G biopsy needle was inserted and advanced till its tip reached beyond the outer needle and entered the placental surface. Stylet of the sampling needle was removed and 30 ml sterile plastic syringe with 5 ml of cultured medium was attached to its hub and suction pressure was applied while the sampling needle was moved up and down into the placenta to cut through the villi for 15 to 20 seconds. The needle was then removed while still maintaining the

suction pressure. The contents of the sampling needle were then immediately flushed into a sterile petri dish and examined under the dissecting microscope. If the sample was inadequate the procedure was repeated. A maximum of 3 such aspirations were done. The procedure was considered a failure if no sample was obtained. The outer needle was removed after replacing the stylet at the end of the procedure. Fetal heart rate and any hematoma over the placenta were noted after the procedure. Instruments and procedure of TACVS are shown in Figs. 5and 6 respectively.

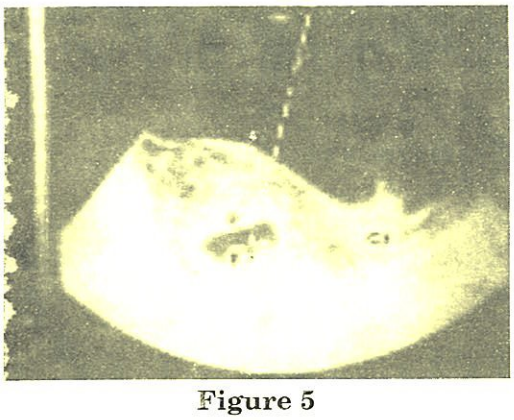

Figure 5

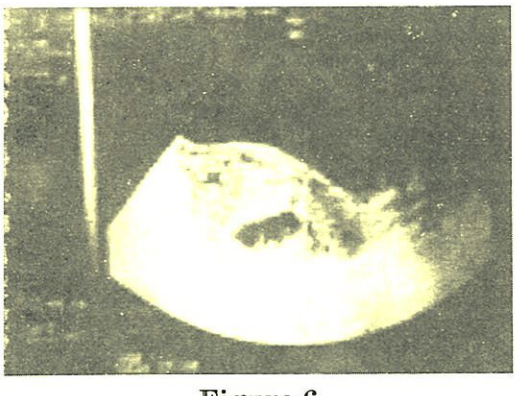

Figure 6

Fetal blood purity was assessed by Kleihauer- Betke test. When only fetal cells were seen in at least 50 low power field of each slide the sample was considered to be pure. Disparity of fetal and maternal blood group +Rh status, difference in hematocrits from the mother were noted. For **Fetal skin biopsy** (FSB) the samples were fixed in formalin (light microscopic examination) and in 2.5% glutaraldehyde buffer for transmission electron microscopic examination.

Tumour specimens (16) and ascitic fluid (10) were collected in tissue culture medium M199. Cultures were treated with drugs (16 different combinations of 2-5 drugs). The dose of the drug also varied according to the combination. The *in vitro* drug effect was evaluated by recording the degree of survival of malignant cells under the microscope. The less the cell survival, the more was the drug effect. Patients were treated with the drugs found most effective in the *in vitro* test. Therapeutic evaluation of effectiveness of Cimetidine, Cyperotermeacetate, Aldactone, and OCP in **the treatment of hirsutism** was done. Total body hair scoring was done by the method of Ferriman and Gallaway. Relevant biochemical investigations and US were done to establish the diagnosis. The study group was first given placebo for 3 months and then Cimetidine in the dose of 1400 mg or Cyperothone Acetate or Aldactone or OCP was given from d 5 – 25 of the menstrual cycle.

Uterine balloon therapy was evaluated in three groups of patients

Group I Hysterectomy specimens (6), Group II Patients with DUB undergoing hysterectomy (6) and Group III Patients with DUB treated with uterine Balloon therapy (20). Group III patients were followed up at 1 & 6 month, 1, 2 & 4 year and > 6 years. At each visit patients' history was taken and clinical examination was done followed by TVS to note endometrial thickness, hemato or pyometra, ovarian or any other pathology was noted.

Discussion

The results of percutaneous ultrasound guided fetal cord blood sampling revealed that the technique was successful in 62.1%, 32.4% and 5.4% of the cases after the 1st, 2nd and 3rd attempt respectively in the MTP cases (experimental group). There were 2 cases of failure at 15 wks and 1 at 16 wks. All the failures occurred at the initial stages (7.5%). Weiner (1987) reported a failure rate of 4.2% after 2 years of experience. The success rate increased to 92.5 % in the experimental group after the second and third attempts. Weiner (1987) reported a success rate of 60% after the first attempt and 95 % with 3 or less needle insertions.

However in the diagnostic cases (40) cordocentesis was successful in 60%, 25% and 15% following the 1st, 2nd and 3rd attempts. The success rate increased to 85 % after the second attempt and 100% after the third attempt.

Change in the plane of the ultrasound focus following maternal movement or uterine contraction or fetal movement, and at times the umbilical cord slipped away from the needle tip

Fetal blood sampling : MTP cases Experimental group

POG weeks	No	Failures	No of attempts		
			1	2	3
15	2	2	_	_	_
16	6	1	2	2	1
17	8	_	5	2	1
18	12		8	4	_
19	6	_	4	2	_
20	6	_	4	2	_
Total	40	3	23	12	2
%	_	7.5	62.1	32.4	5.4

thus leading to delay in procedure and increase in the number of attempts which contributed to the initial failure rate. Though the procedure could be performed at all the positions of placenta but sampling from a posterior placenta was difficult. It was also difficult in cases when the period of pregnancy was less than 17 weeks due to the smaller diameter of the vessel. In our pilot study (not included) we observed that 25G needle got easily bent hence it was discarded. There were more complications (bleeding from the cord and bradycardia) with 20 G needle hence we used 22 G only in all our cases. Ludomirski and Weiner (1988) stated that 22 G is the most widely used needle. We punctured umbilical vein because it is wider and as a straight course. Part of the aspirated blood was injected into the vessel to identify if the tip of the needle was in the umbilical vessel to note the turbulence on the ultrasound monitor and this insured that the needle tip was in the vessel. We used free hand and fixed needle guide technique. The immediate complications after FBS noted were bleeding from the cord and bradycardia.

Complications of FBS

Complications	MTP (1986-89)	Dx cases (1988-91)	Daffos (1985)	Weiner (1987)
Bleeding from the cord	35.1% (13)	40% (16)	41%	42%
Bradycardia	10.8 %(4)	10%(4)	7%	3.1 %

Puncture of umbilical artery and use of large bore needle cause more and longer bleeds. However hemostasis is rapid and effective due to the elasticity of the Wharton's jelly and rapid coagulation mechanism.

Slow heart rate lasting for a longer time is an ominous sign and was reported in the only procedure related intrauterine death in the Daffos series (10). None of the fetuses, or cord, or placenta had shown any marks of trauma nor was there any evidence of maternal trauma after the procedure. Fetal risks depends upon the indication of the procedure. Two deaths occurred within 1 week of FBS in severely hydropic fetus and one in a case of severe IUGR. Fetal loss of 1% is reported by Nicolaides. They lost 4 fetuses 1-6 weeks following the procedure in minimally affected hydropic fetuses.

Fetal blood sampling was very useful in providing the correct status of the fetus in Rh isoimmunised pregnancies thus timely LSCS could save majority of the cases. It is an accurate method of assessing fetal anemia, its utility in cases of NIHF is illustrated by our case when there was fetal anemia and normal

karyotype. This prompted us for intrauterine fetal therapy at 25 weeks and the baby could be saved.

Cordocentasis is being increasingly used for

I. Blood Disorders

a. Hemoglobinopathies (thalassemia, sickle cel anemia)

b. Coagulopathies

c. Thrombocytopenia

d. Immunodeficiency

II. Infections

a. TORCH group

b. ? AIDS

III. Metabolic disorders

IV. Chromosomal anomalies

V. Prenatal hypoxia

VI. Red cell immunization (Rhesus, Kells)

Diagnostic cordocentesis – 40 Indications	
Rh isoimmunised	12
NIHF	5
Congenital malformation	8
IUGR	5
Down syndrome	6
Ch. Anomaly	2
Rubella	1
Ventriculo megaly	1

With the ready access to the fetal circulation, cordocentesis has become a reliable tool in the *in utero* management of severe red blood cell isoimmunization (6) and thrombocytopenia (11). FBS holds great promise in the newly conceived and ever expanding field of fetal medicine. It is hoped that with further research it will provide insight into fetal physiology and pathology in different fetal disorders. Though the procedure is simple, it is safe only in experienced hands and hence it is best confined to specialized referral centers concerned with fetal disease and therapy.

Fetal skin biopsy

At present there is no effective treatment for several devastating hereditary skin disorders as well as genetic disorders associated with skin abnormalities. The only logistic approach is prenatal diagnosis and elimination of the affected fetus. Fetal skin biopsies have been performed under fetoscopic guidance (7). But this is associated with high fetal loss. We evaluated a minimally invasive us guided technique of fetal skin biopsy first in the experimental case (15) and our success rate was 50%. This was because we limited our procedure to 10 minutes to minimize the risk of infection and complications and the learning curve. The success rate increased in our later cases. In all cases 4-6 samples were taken to ensure adequacy of the sample. We took biopsy from the fetal scalp because head is large and relatively immobile. Histopathological examination of the fetal skin revealed that keratin

was not present in biopsies taken before 18 weeks. Keratinisation and maturity of the skin was observed in samples taken after 18 weeks and hair follicles were recognized. Thus fetal skin biopsies should be taken after 18 weeks of pregnancy. Our success rate in diagnostic cases was 100%. We could exclude occulocutaneous albinism, diagnose epidermolysis bullosa and sjogren syndrome and in a unique case of absent sweat gland, pain and thermal sensation.

Fetal skin biopsy indications	
Spedermolysis Bullosa	4
Sjogren's syndrome	1
Occulocutaneous albinism	7
Prev child with absent sweat gland, thermal & pain sensation	1

Transabdominal Chorion Villus Sampling

Indications : Down Syndrome, Thalasemia, Conginital adrenal hyperplasia, DMD, Advance maternal age, Hunter syndrome, Fragile syndrommme, others 25 – 1st trimester, 15- 2nd trimester

Transabdominal chorion villius sampling –100

Management of dysfunctional uterine bleeding has come a long way. In the era of minimally invasive techniques, the Uterine Balloon Endometrial Ablator (TBEA) is a boon to gynaecologists. It is a new method to treat menorrhagia. The endometrium is destroyed using heated 5% Dextrose (5%DW) filled within a latex balloon. Before it is used in patients with dysfunctional uterine bleeding, it was imperative to confirm that the heated liquid under pressure in the balloon did not cause any damage to the surrounding structures. And to know the safety limits of temperature and pressure beyond which rupture of balloon, perforation of uterus and damage to surrounding tissue might occur. Neuwirth *et al* (12) performed pre- clinical safety studies which indicated that rupture of the uterus did not occur with balloon pressure upto 200 mmHg. Thermal injury was evaluated first in specimens of meat. Similar thermal coagulation effects were produced later in specimens of human uterus. We performed UBT on 6 uteri after hysterectomy and in 6 cases prior to hysterectomy. There was no perforation or rupture of uteri. Thermal damage was visible as a zone of erythema in the endometrium and superficial myometrium including cornual areas. It measured 5.4 mm (mean). The range of destruction varied between 3-9 mm. It is of interest to know how much of the endometrium is damaged and what changes are evident in the endometrial cells. Three uterine specimens were studied immediately after TBEA and two after five months of TBEA. Our study revealed early changes in the form of hemorrhage, congestion, eosinophilic infiltration and necrosis. Basal endometrium and areas of hyalinization were evidence of late

changes. The change of temperature in the nearby organs was negligible (0°C-1°C). There was no evidence of uterine perforation or rupture. In the present study only those women who were more than 25 years of age with uncontrolled excessive bleeding due to benign causes, family complete, uterocervical length (UCL) 12 cms and less (range 7-10 cms), benign endometrial histology and normal papanicoloau tests were included in the study. Thus from our study, it can be said that uterine balloon ablator is a safe, simple and effective procedure to treat menorrhagia. The success rate achieved by TBEA and by other endometrial ablation procedure (Nd: YAG, electroresection, radiofrequency thermal ablation) are similar (80-90%). However, its high cost is a deterring factor for it to be a routine procedure in India. We need to develop ingenious and cheaper alternatives so that this procedure may be available to the population at large.

Conclusion

Advances in the field of obstetrics and gynecology have given birth to the concept of the unborn fetus as a patient accessible for diagnosis and therapy in India. With the development fetal blood sampling, chronic villous sampling and fetal skin biopsy we are able to assess and evaluate the fetal well being much better.

Fetal blood sampling

In experienced hands it is a invaluable tool in assessing fetal well being and a reliable guide in planning fetal management in the presence of disorders such as Rh isoimmunisation, non immune hydrops, congenital malformations, infections and rapid karyotyping. It is an OPD procedure that can be done from 17 weeks onward till late third trimester.

Fetal skin biopsy

It is a sophisticated technique requiring considerable experience. It is an OPD procedure and should be done after 18 weeks of pregnancy. Occuloaltaneous albinism could be excluded by demonstrating melanogenesis in the fetal skin by electronic microscope. And various other fetal skin disorders could be diagnosed.

Transabdominal chorion villus sampling

It is an optimal method and should replace trans cervical method.

In the last two decades obstetrics and gynaecology has rapidly grown to be one of the most sought of specialties. But the need of the hour is to treat and manage our patients on the basis of evidence based medicine.

Acknowledgement

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References

1. Rodeck, CH, and Nicolaides, KH (1983) Ultrasound guided invasive procedure in obstetric. *Clinics in Obstet. Gynae* **10:** 515-539
2. Ward, RHT, Modell, B Fairweather, DVI, *et al* (1981). Obstetric outcome and problems of mid trimester fetal blood sampling for antenatal diagnosis. *Br. J. Obstet. & Gynaecol.* **88**: 1073-1080.
3. Daffos F, Capella – Pavlosky M, Forestier F (1983). A new procedure for fetal blood sampling in utero : preliminary results of 53 cases. *Am. J. Obstet & Gynaecol* **146**: 985.
4. Berkowitz,RL, Chitkara,U, Wilkins, I, *et al* (1987): Technical aspects of intravascular intrauterine transfusion : Lessons learned from thirty three procedures. *Am.J. Obstet. & Gynaecol.* **157**:4-9
5. Grannum PA, Copel,JA, Plaxe,SC (1986): Inutero exchange transfusion by direct intravascular injection in severe erthroblastosis fetalis. *New Engl. J. Med* **314**:1431-1434.
6. Rodeck, CH, Nicolaides, KH, Warsof, SL, *et al* (1984). The management of severe Rhesus isoimmunization by fetoscopic intravascular transfusion. *Am. J. Obstet. Gynaecol.*,**150**: 769-779.
7. Eady, RAJ, and Rodeck, CH (1984). Prenatal diagnosis of disorders of the skin – In Prental diagnosis: Proceedings of the eleventh study group of the royal college of Obstetricians & Gynaecologists Sept 1983,1984: 147-158.
8. Rodeck, CH, and Nicolaides, KH (1983) Ultrasound guided invasive procedure in obstetric. *Clinics in Obstet. Gynae*, **10:** 515-539.
9. Kazy,S, Rovsky IS & Bakharev, FA (1982). Chorion biopsy in early pregnancy a method for early diagnosis of inherited disorders. *Prenatal diagnosis* **2**:39-45.
10. Daffos F, Capella – Pavlovsky, M., Forestier, F. (1985): Fetal blood sampling during pregnancy with use of needle guided by ultrasound: A study of 606 consecutive cases. *Am. J. Obstet. Gynecol.* **153**:655.
11. Daffos F., Forestier,F.,(1984) : Prenatal treatment of alloimmune thrombocytopenia. *Lancet*: **ii**:632.
12. Neuwirth RS, Duran AA, Singer A, Macdonald R, Bolduc I (1994): The endometrial ablator : A new instrument . *Obstet Gynecol* **83**: 792-796.