MATERIALS AND METHODS
Materials and methods

Two different hospitals situated in Ahmedabad city namely B.J. Medical College, Civil Hospital and N.H.L. Municipal Medical College, V.S. Hospital were selected as venues for the study. On the whole 60 expectant mothers were selected and information on the socio economic background, hemoglobin level, food habits, maternal history, obstetric performance and pregnancy outcome details were collected.

The present study involved use of 60 freshly collected placenta from labour room and also from the operation theatre after caesarian section from the department of Obstetrics & Gynaecology, Smt. N.H.L. Municipal Medical College, Ellisbridge, Ahmedabad. All the placentas collected from labour room weighed from 440 gm to 520 gm were utilized for present study. Before collection placenta were carefully examined for other gross abnormalities like, marginal insertion of umbilical cord, infarction on the maternal surface of the placenta, accessory lobes and abnormal placenta were not selected for the present study.

The study employed by two methods:

1. Injection corrosion cast technique
2. Meticulous dissection

Injection corrosion cast technique

Introduction

Leonardo Da Vinci (1452-1519) as quoted by Tompsett (1956) was probably the first one to study the tubular organs by preparing their casts, to get the three dimensional picture of the organ. He used a low melting point object like wax for this purpose. It was replaced in the late 19th century by low melting point metal alloys, but its use was discontinued because its weight ruptured thin walled structures. Plastic rubber
solutions and resins replaced these metals in the first half of the 20th century. Resins and plastic congeners are still remain favorite choices for many, who employ the corrosion cast technique.

**Material used**

- Cellulose acetate butyrate (CAB) granules
- Potassium hydroxide pellets (KOH)
- Solution of acetone
- Phosphate buffer
- Heparin
- Procaine
- Diluted H₂SO₄
- Plastic syringes
- Different bored plastic canula
- Normal saline and other required material like, air-tight glass bottles, glass rods, glass jars, dissecting instruments, threads for ligature.

**Principle**

Corrosion cast technique employs a filling of hollow tubular structure with a non-sticky material which was dissolved in an appropriate volatile solvent. The uniformly prepared solution is then injected into the organ. The volatile liquid evaporates gradually, and the solutes settle down inside the tubes (blood vessels) of the organ forming a solid permanent cast. The unwanted tissue then washed away by using a suitable corrosive agent (KOH), which does not corrode the cast. Acids and alkalis are commonly used as corrosive agents to remove unwanted soft tissue from the specimen.

The cast material (CAB) was dissolved in an appropriate solvent (Acetone) which is volatile, and injected into the arterial tree. The solution occupies the arterial tree and solidifies there because of
evaporation of the volatile solvent and forms permanent cast. The parenchyma digested totally to display the arterial tree. The resultant specimen gave complete three-dimensional picture of an arterial tree.

**Preparation of the solution**

The present study employed the use of cellulose acetate butyrate (CAB) granules. Earlier studies of corrosive casting used cellulose acetate but now CAB is preferred because cellulose acetate did not form a uniform solution which could not give uniform permanent cast and was sticky, preventing uniform injection of the solution.

We used 50% solution of the Cellulose acetate butyrate for the purpose of injection. The granules of CAB were dissolved in a solution of acetone in a ratio of 1:2 and kept in air-tight glass chambers for 72 hours, for complete dissolution of the CAB granules. It was then stirred to make uniform solution and kept ready in different colors, safely in air-tight glass bottles. Plastic colors were used for to make colorful solution, red for artery and blue for vein.

**Collection of the specimen**

Sixty mothers with uncomplicated pregnancy were selected from indoor patients of Gynaecology and Obstetrics Department of V.S. Hospital (Smt. N.H.L. Municipal Medical College, Ellisbridge, Ahmedabad) and Civil Hospital (B.J. Medical College, Asarwa, Ahmedabad). The age range of these mothers varies from 20 years to 38 years, belonged to middle class family; income per family being Rs. 5000/- to 10,000/- per month. Mothers were examined clinically (for height, weight, blood pressure, pulse, anaemia, jaundice etc.) along with recording of their medical history (history of past illness, history of previous child birth etc). Their investigation reports were checked (blood sugar, urea, creatinine, hemoglobin levels, urine for albumin, pus cells).
In this method freshly collected specimen from the labour room of the Department of Obstetrics and Gynecology were preferred. These specimens, before injection of cast material were thoroughly rinsed with normal saline to remove any blood clots. The size, shape, surface area, weight of placentas were noted along with the inspection of marginal veins for any thrombus; the number of cotyledons, condition of membranes, presence of infarction, calcification and site of insertion of umbilical cord were noted.

After delivery placentas were collected for morphological study of vascular architecture of the umbilical artery. At the time of collection, placentas were carefully examined for the presence of any abnormal branching pattern of the umbilical vessels. Amniotic membrane was carefully removed from the surface of the placenta and umbilical artery and vein were traced up to their termination. The main trunk of the umbilical vessels was examined carefully to know about the mode of terminal branching and any variations.

The specimen was then decapsulated on the maternal surface and then immersed in a mild solution of KOH (5%) and kept in a glass jar for about 24 hours. This resulted in the softening up of the parenchymal tissue of the placenta. The umbilical vessels traced from attachment of umbilical cord on fetal surface to the maternal surface of the placenta. The terminal branches were then traced right from the maternal surface by carefully dissecting and removing adjacent parenchyma bit by bit and then keeping it under constant stream of running water to wash away the delicate parenchyma. The resultant specimen displayed necessary details.

Care was taken to preserve the external features like placental septa, cotyledons and attachment of the umbilical cord. This in particular, was done to look for any avascular planes in relation to cotyledons.
Infusion of the umbilical artery with CAB solution and micro-dissection of the intervening tissue produced a cast of the vasculature, which can give idea about branching pattern of the umbilical vessels. Out of sixty human placentas collected after normal delivery from Obstetrics & Gynaecology department of V.S. Hospital and Civil Hospital, Ahmedabad, only fifty-five placentas were used for corrosive cast study and rest for morphological analysis only. Suitable placentas were immediately perfused with phosphate buffer which contained 1000 IU/l heparin as anticoagulant and 1% procaine for vasodilatation, either through fetal branches of the umbilical artery and vein.

**Procedure of injection**

Before infusion, umbilical artery and vein were dissected through fine dissection technique. The main trunk of the umbilical vessels identified, cleaned and a tight fitting plastic intravenous cannula was introduced through it and was fixed in place by ligature. Plastic syringes, filled with normal saline were fitted into the cannula. The umbilical arterial and venous tree was thoroughly rinsed with normal saline to remove any blood clots. The venous end was inspected until clear solution started to come out of it, a sign which indicated that the veins were cleared of any blood clots. In these ways umbilical artery and vein were cleaned. Subsequently liquid plastic compounds of CAB in acetone were freshly prepared and were stirred to make a uniform solution before injection.

The syringe was filled with the red solution and fitted into the cannula for umbilical artery and blue solution for the umbilical vein. The plastic mixture was freshly prepared and cooled prior to instillation, thus delaying polymerization for approximately 10 min. The plastic was instilled via a syringe under manual pressure at a flow rate of approximately 5 ml/min. The procedure continued until the organ became tense and
resistance felt while injecting. The umbilical arteries and vein were clamped after instillation to prevent efflux of plastic and to maintain the instillation pressure within the system.

**Setting up of the injected mass**

The injected specimen was then transferred to the mixture of formalin and saline and let it settle for 48 hours to help setting up of the injected mass. The specimen was then palpated to make sure that solution had settle down to form a solid cast, because of the evaporation of the volatile acetone.

**Corrosion/ Maceration**

The placentas were excised and placed in a water bath at 20 °C for 30 min, followed by immersion in a water bath at 80 °C for several hours to allow hardening of the plastic. An alkali, potassium hydroxide (KOH) was used for the maceration of the unwanted parenchyma. 40 % solution of KOH was prepared fresh and kept ready to use. Corrosion was performed over several days by alternating immersion of the plastic-instilled tissue in 40% KOH and distilled water, both at 60 °C. Once the washing was complete the vessels were displayed. Finally, the casts were examined to observe the branching pattern of the umbilical vessels. The specimen was allowed to cure for 24 hours before being placed in a bath containing KOH in water (4% wt/vol) to digest the tissue. After marked digestion of both uterine and fetal tissue had occurred, placental vascular casts were removed from the initial digestion bath and placed in fresh KOH in water to finish digestion of the remaining placental membranes before being rinsed with copious amounts of water and allowed to dry.

**Dissection technique**

Dissection method was undertaken to see the details of arrangement of the cotyledons, arrangement of the branches of the
umbilical artery and vein and relation of the avascular planes to the long axis of the organ and in relation to the cotyledons if any.

The intra-placental distribution of the umbilical vessels was carefully observed after fixation of the casting material. This included confirmation of the existence of segments, number of cotyledons, evidence of any avascular planes and its relation to the long axis of the organ and any external features like accessory lobes, arrangement of the cotyledons and anastomosis between the umbilical artery and vein.

The prepared specimen either cast or the dissected one was then photographed from different angles. Each specimen was numbered, with indelible ink on a tag of cotton cloth and detailed sketch of each placenta was prepared on the graph paper to measure the amount of area of the parenchyma supplied by each cotyledonary artery. Tables were prepared for comparative study. Pattern of nomenclature for different segment were decided.