DISCUSSION

Smooth chorion
Fetal circulation
Intervillous space
Amnion
Chorionic plate
Stump of main stem villus
Branch villi
Main stem villus
Placental septum
Decidua basalis
Myometrium

Umbilical vein
(O$_2$-rich blood)
Umbilical arteries
(O$_2$-poor blood)

Amniochorionic membrane
Decidua parietalis
Smooth chorion
Amnion
Stump of main stem villus

Anchoring villus
Cytotrophoblastic shell
Endometrial veins
Endometrial arteries

Intervillosus space
Fetal circulation
Branch villi
**Discussion**

**Selection of placentas for study**

In this study, the specific method of placenta selection allowed comparable material to be obtained from pregnant women. The placentas located in the tips of gravid and in non-gravid uterus horns, as well as accessory placentas, which are generally smaller than circum-conceptional ones, were excluded. The trans-placental exchange depends upon 2 substance absorbing surfaces of the placental villosity, the outer villous trophoblast surface and the inner vascular endothelial surface. The efficiency of substance exchange is improved by the increasing area of both these surfaces per volume of placenta.

**Morphological study**

Morphological findings of present study compared with those of earlier studies. Mean birth weight of baby in present study is 2.735 kg which is 2.64 kg in Udainia A\textsuperscript{27} (2001) and 2.8 kg in Mazumdar S\textsuperscript{32} (2005). Mean weight of placenta in present study is 474.1 gm, which are 495.0 gm in Udainia A\textsuperscript{27} (2001) and 485.85 gm in Mazumdar S\textsuperscript{32} (2005). Mean placental surface area in present study is 264.43 sq. cm which is 242.56 sq. cm in Udainia A\textsuperscript{27} (2001) and 265.15 sq. cm in Mazumdar S\textsuperscript{32} (2005). Mean placental volume in present study is 578.91 cm\textsuperscript{3} which is 612.98 cm\textsuperscript{3} in Mazumdar S\textsuperscript{32} (2005).

Mean ratio of fetal/placental weight in present study is 5.763 which is 5.89 in Mazumdar S\textsuperscript{32} (2005). Mean number of cotyledons in present study is 18.967 which is 17 in Mazumdar S\textsuperscript{32} (2005). In present study, 8.33 % of placenta shows marginal insertion of the umbilical cord,
in study carried out Mazumdar S\textsuperscript{32} (2005) 5.2 % of placenta shows marginal insertion of the umbilical cord.

Table 4: Comparison of Morphological findings of present study with other studies carried out on placenta

<table>
<thead>
<tr>
<th></th>
<th>Present study (2008)</th>
<th>Udainia A\textsuperscript{27} et al. (2001)</th>
<th>Mazumdar S\textsuperscript{32} et al. (2005)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean wt. of baby (in kg)</strong></td>
<td>2.735</td>
<td>2.64</td>
<td>2.8</td>
</tr>
<tr>
<td><strong>Mean wt. of placenta (in gm)</strong></td>
<td>474.1</td>
<td>495.0</td>
<td>485.85</td>
</tr>
<tr>
<td><strong>Mean placental surface area (sq. cm)</strong></td>
<td>264.43</td>
<td>242.56</td>
<td>265.15</td>
</tr>
<tr>
<td><strong>Mean placental volume (in cm\textsuperscript{3})</strong></td>
<td>578.917</td>
<td>NA</td>
<td>612.98</td>
</tr>
<tr>
<td><strong>Ratio of fetal/placenta wt.</strong></td>
<td>5.763</td>
<td>NA</td>
<td>5.89</td>
</tr>
<tr>
<td><strong>No. of cotyledons</strong></td>
<td>18.967</td>
<td>NA</td>
<td>17</td>
</tr>
<tr>
<td><strong>Marginal insertion of umbilical cord in %</strong></td>
<td>8.33</td>
<td>NA</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Damania KR\textsuperscript{77} et al (1989) had studied sixty placentae of hypertensive disorders of pregnancy and had reported that birth weight, placental weight and feto-placental ratio were less in hypertensive cases than in the normotensive controls. It corresponds to the findings of Thomson AM\textsuperscript{78} et al (1969). In our study, the birth weight shows an increase with the ascending surface area of the placenta. The feto-placental ratio is found to be almost constant in all cases. This is in confirmity with the findings of Younoszai and Haworth\textsuperscript{79} (1969) who have
reported that the placental weight and size are directly proportional to the birth weight. Perceival\textsuperscript{80} (1980) has reported that in 73 percent of cases, the site of insertion of umbilical cord is eccentric in position (somewhere between the centre and edge of the placenta). In present study, 83.33 \% placenta having central attachment, 8.33 \% having intermediate attachment and 8.33 \% having marginal attachment of the umbilical cord. In our analysis, it was observed that birth weight, placental weight, surface area of the placenta and feto-placental ratio of the marginal cord are less than those of the central cord insertion. In our study, the eccentric attachment of umbilical cord percentage was seen is 16.66\%, which is lower than as noted by Perceival\textsuperscript{80} (1980).

Woods and Malan\textsuperscript{81} (1978) have studied 940 placentae and found no correlation between the birth weight and the site of cord insertion in normal term infants. However, in the present study, even in normotensive mothers the marginal attachment of umbilical cord was correlated with low birth weight babies. Besides the normotensive mothers, in previous studies shows that even in cases of mild, moderate and severe hypertensive cases, the marginal attachment of umbilical cord are related to low birth weight, most commonly noticed in the severe hypertensive sub-group. These findings are similar to those reported by Shanklin\textsuperscript{82} (1970), who after studying 5000 placentae, observed a high degree of correlation between anomalous cord insertion and low birth weight.

The association between marginal attachment of the cord and low birth weight is statistically significant. The low birth weight may be explained by an altered distribution of fetal blood in the placenta as a result of different modes of arrangement of intracotyledonary vessels of placentae of complicated pregnancy (Rath\textsuperscript{83} et al 1994). This vascular
arrangement may be hampering equal distribution of blood flow in the placenta, increasing the risk to the mother and fetus. Sonographic study could also show clearly the site of insertion of the umbilical cord of the placenta and in hypertensive mothers, it was mostly marginal. It confirms the observations of Pretorius\textsuperscript{21} et al (1996) and Di Salvo\textsuperscript{22} et al (1998). In case the ultrasound report reveals marginal attachment, it is advisable to get it confirmed by colour doppler imaging, if possible. In conclusion, the present study reveals the method of precise location of umbilical cord by calculation of insertion percentage. It is noticed that the marginal insertion is associated with hypertension and that it can be diagnosed during antenatal check up by available technique to further strengthen the proposed precautions to be taken during and after labour.

Thomson AM\textsuperscript{78} et al (1969) and Younoszai and Haworth\textsuperscript{79} (1969) reported that the placental weight and size are directly proportional to the birth weight. Perceival\textsuperscript{80} (1980) observed that the eccentric attachment of the umbilical cord was most common in normal placenta. Shanklin DR\textsuperscript{82} (1970) noticed velamentous or marginal type of cord insertion in infants weighing less than 2500 gms. Younoszai and Haworth\textsuperscript{79} (1969) who have reported that the placental weight and size are directly proportional to the birth weight.

The gross anatomic features of placentae e.g infarcted areas, calcified areas and marginal insertion of the umbilical cord in the study group show significant increase in value (p>0.01) when compared to that of the control group. This is in concurrence with the findings of Fox (1967) and Udainia et al (2004) who had observed a similar increase in the incidence of placental infarction with severity of toxaemia. Also,
Pretorius (1996) reported cases of marginal insertion of placenta in about 42% cases of pregnancy induced hypertension.

**Corrosive cast study: Fetal vasculature**

The structural organization of the chorionic vasculature was analyzed from cast models of full-term placentas. The models were generated by the corrosion technique using a CABG polymer mixture. The low viscosity of the polymer mixture enabled manual filling of the blood vessels, including the capillary region. The polymer mixture was colored differently for the arteries and vein to distinguish between their branches. The resulting cast models revealed the capillary system stemming from the IP vessels, which penetrate into the placenta from the chorionic plate. These capillary trees were similar to structures obtained by other techniques (Kaufmann et al. 1985), which implies that our casting technique was reliable.

The polymeric casts of the fetal vasculature of full-term placentas provided information on the geometry of the umbilical, chorionic and IP vessels. Two umbilical arteries were dissected from the substance of umbilical cord by meticulous dissection.

The cast models of present study demonstrated very well the Hyrtl anastomosis between the umbilical arteries in the vicinity of the umbilical cord insertion into the placenta (Arts, 1961; Raio et al. 1999, 2001; Ullberg et al. 2001). We observed that two umbilical arteries anastomosed (Hyrtl anastomosis) with each other deep to the fetal membrane before dividing in cotyledonary branch. An anastomosis between the two umbilical arteries, located approximately within 5-10 mm from the placental cord insertion. Out of 55 normal placentas used for corrosive casting, in 50 cases there was one anastomosis between the
umbilical arteries, in three cases there were two, in two the anastomosis was absent. Ullberg U\textsuperscript{59} et al (2001) studied 67 normal placentas, in 60 cases there was one anastomosis between the umbilical arteries, in one case there were two, in four the anastomosis was absent, and another two cases had a single umbilical artery. Gordon Z\textsuperscript{85} et al (2007) observed that Hyrtl anastomosis is present in all 15 casts, which connects the umbilical arteries within the placenta at a distance of 5 mm from the cord insertion. Raio L et al (1999\textsuperscript{54}, 2001\textsuperscript{58}) observed that an anastomosis between the two umbilical arteries, located approximately within 3 cm from the placental insertion, has been previously described at delivery and in utero. They concluded that the Hyrtl anastomosis may act as a pressure-equalizing system between umbilical arteries. This supports the hypothesis that the Hyrtl anastomosis plays an important role when the placental territories supplied by the umbilical arteries are different in size. The anastomosis was represented by a vessel, a fenestration or coalescence of the umbilical arteries.

The branching pattern of present study shows that the chorionic vasculature exhibits a combination of the dichotomous and monopodial patterns, as in other biological branching systems (e.g. pulmonary bronchi). Observation of all 55 placental casts of our study does not reveal a pure dichotomous or monopodial branching pattern within the chorionic plate. In present study 50 placentas of central cord insertion (Fig. 10), the blood vessels bifurcate mostly in a dichotomous pattern with a few monopodial ramifications from some dichotomous generations and in 5 placentas of intermediate cord insertion dichotomous pattern is dominant with presence of monopodial pattern. These results support previous observations that the network of chorionic blood vessels represents a mixture of dichotomous and monopodial branching patterns.
(Gordon Z et al (2007)). In the present study we prefer the more accurate terminology of 'dichotomous' and 'monopodial' branching patterns.

As observed by Gordon Z et al (2007), the placental casts demonstrated that umbilical cord insertion was located anywhere between the center and the margins of the placenta. For a central insertion the dichotomous pattern was dominant, whereas for a marginal insertion the monopodial pattern was dominant. In the more frequent cases of cord insertion neither in the center nor in the margin, the branching structure was a mixture of dichotomous and monopodial patterns. These results support previous observations that the network of chorionic blood vessels represents a mixture of dispersal and magistral branching patterns (Benirschke & Kaufmann, 1995).

Verma R et al (2010) studied Cellular Changes in the Placenta in Pregnancies Complicated with Diabetes. They observed that the dispersal vascular pattern of chorionic blood vessels of placenta was more frequently observed in the placentas of GDM controlled on diet and also in control group while magistral pattern was more common in the placentas of GDM controlled on insulin. Usually, the dispersal vascular pattern the chorionic blood vessels of placenta are more frequent than the magistral pattern. The site of attachment of Umbilical cord Eccentric or central is most of the placentas except in one case in which there was marginal insertion of placenta.

T. Peker et al (2006) carried out general assessment of the microanatomical structure obtained by corrosion cast revealed that the umbilical artery gave off 2-4 primary branches (median number of primary branches of the umbilical artery in both normal and pre-eclamptic placentas was 3) while just entering the placenta. Each primary branch
gave off 4–9 secondary branches (the median number was 7 and 6 in normal and pre-eclamptic placentas, respectively). These branches showed a radial course within the placenta. No significant change was found between normal and pre-eclamptic placentas in terms of diameters and numbers of the umbilical arteries' main, primary and secondary branches ($P > 0.05$). The intraplacental course of the umbilical artery showed a straight course in all the normal placentas while in three of the six pre-eclamptic placentas it showed a tortuous and, in the remaining three, a straight course. Their study also revealed that the umbilical artery gives off primary and secondary branches while entering the placenta. Branching occurred at the level of intermediate villi. While the umbilical arteries showed a straight and radially extending intraplacental course in all normal placentas, they proved to have equally a straight or a tortuous course in pre-eclamptic placentas.

Lee MM, Yeh MN (1983) compared the ultra-structural appearance of the fetal microvasculature of the baboon and human placenta by the latex injection corrosion-cast technique. The main findings based on observations of the SEM appearance of the human placental casts at three-quarter gestation and at term include the following: (1) the arterial vessels do not exhibit a marked tortuosity but do retain a rough surface contour, (2) stem vessels appear to branch at a horizontal plane before dividing into numerous smaller branches, (3) the venous side has straight, wide-calibre vessels with smooth surface contour, and (4) the capillary bed is densely tortuous on the arterial side and less tortuous on the venous side.

In the centrally inserted cord the vessels that branch off the umbilical arteries traverse along half of the placenta diameter, whereas in an intermediate insertion three of those branches traverse along two
thirds of the diameter and rest of two along only about one third of the chorionic plate diameter. These findings are supporting previous findings of Gordon Z85 et al. (2007).

In present study, the chorionic vessels branch through 6-8 generations from the cord insertion towards the margins of the chorionic plate. Measurements of the chorionic vessels showed that the first dichotomous bifurcation of the umbilical arteries was about 1.5 cm from the insertion, and the second one 2.5-4.5 cm from the insertion. Gordon Z85 et al. (2007) noted that the chorionic vessels branch through 6-8 generations from the cord insertion towards the margins of the chorionic plate. Measurements of the chorionic vessels showed that the first dichotomous bifurcation of the umbilical arteries was about 1 cm from the insertion, and the second one 2-4 cm from the insertion.

The vein bifurcated twice immediately after insertion into the placenta, creating four branches over a distance of less than 0.8 cm. The IP vessels entered the placenta at angles of 60-90° to the chorionic plate. These findings are supporting previous findings of Gordon Z85 et al. (2007).

The data measured from all the placentas were used to evaluate a typical network for the chorionic arteries. Since the branching pattern is a mixture of dichotomous and monopodial patterns, we integrated the measured data from all the casts to propose the geometry for a typical network branching off an umbilical artery, as shown in Fig. 11. The Geometric data is compared with the previous study of Gordon Z85 et al. (2007) in Table 5.

Classification of vasculature branching networks is well established in the literature, especially in organs like the coronary arteries of the human heart, which were sorted into two groups (Aharinejad74 et al.
The first group is composed of 'distributing' vessels, which run along the heart borders and convey blood to major zones. The second group is composed of the 'delivering' vessels that branch off from the distributing vessels and enter into every zone of the myocardium to deliver blood at sufficient low velocities that permit gas and nutrition exchange.

Table 5: Comparison of Geometric data of the proposed typical network that branches off the umbilical artery.

<table>
<thead>
<tr>
<th>Segment label</th>
<th>Segment length (cm)</th>
<th>No. of monopodial branches in segment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Umbilical artery</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>1Rt</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>1Rt,2Lt</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>1Rt,2Lt,3Rt</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>1Rt,2Lt,3Rt,4Lt</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>1Rt,2Lt,3Rt,4Lt,5Rt</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>1Rt,2Lt,3Rt,4Lt,5Rt,6Lt</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>1Rt,2Lt,3Rt,4Lt,5Rt,6Lt,7Rt</td>
<td>1.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

A similar architecture exists in the placenta where the chorionic blood vessels convey blood from the umbilical cord across the whole chorionic surface of the placenta to perfuse blood into the whole volume of the placenta. However, the requirement for homogeneous blood supply to all regions of the chorionic plate to ensure efficient exchange of metabolic products within all the cotyledons dictates the anthropometry of the chorionic vasculature, depending on the location of the cord insertion. Thus, the pattern is not like that of the conveying (or distributing) vessels on the heart border, but a combination of both.
conveying and delivering vessels. In case of a central cord insertion, the distance to the chorionic plate margin is only one half of the placenta diameter and a dichotomous branching pattern may be the easiest way to traverse most of the chorionic plate with a minimal number of generations. A few monopodial ramifications coming off the first and second dichotomous branches ensure perfusion of the areas in between. In intermediate and marginal cord insertion, the distance to the opposite margin may be larger than central cord insertion. In this case, the most efficient way to supply blood would be a monopodial branching pattern in which the diameter of the main conducting vessel is almost constant, with minimal variation in the resistance to blood flow. However, in a pure monopodial pattern a relatively large area perpendicular to the main trunk will not be adequately perfused. Thus, the first 2-3 generations of the umbilical arteries branch in a dichotomous pattern and then follow a more monopodial pattern. It should be noted that Benirschke & Kaufmann (1995) were aware of the fact that the organization of the chorionic vasculature is consistent, not random, and dependent on the umbilical cord insertion, but they could not explain the physiological reasons.

Placental development and feto-placental angiogenesis are critical for successful gestation and have been studied comprehensively in relation to fetal pathologies such as pre-eclampsia, intrauterine growth restriction (IUGR) and abruption (Chaddha et al. 2004). It is well established that feto-placental circulation becomes functional, with a closed loop of blood circulation, when the fetal heart starts to beat at about 5.5 weeks of gestation (Salafia & Maas, 2005). There is also strong evidence that the vasculature of the chorionic plate is functionally established by 12 weeks of gestation (Boyd & Hamilton, 1970). It is generally agreed that the normal placenta shape at term is ovoid with
cord insertion near the disk center, but it is unclear how and why an asymmetric placenta with a marginal cord insertion is developed. However, there is evidence that it is related to early compensation due to abnormal intrauterine oxygen gradients and nutrient availability, which induce placental regression from what was supposed to be a normal placenta with a central cord insertion (Jauniaux\textsuperscript{91} et al. 2003; Chaddha\textsuperscript{88} et al. 2004; Salafia & Maas\textsuperscript{89}, 2005).

The chorionic plate vessels constitute the 'high-capacity low-resistance' of the feto-placental vasculature that links the umbilical vessels to the actual sites of oxygen and nutrient exchange in the placental villi. Since the feto-placental vasculature takes up to 50% of fetal blood volume (Adamson\textsuperscript{92}, 1999; Salafia & Maas\textsuperscript{89}, 2005), the function of the chorionic plate vessels is crucial in fetal development. Many studies were conducted to explore the role of angiogenic growth factors in the process of placental morphogenesis and their correlation with pregnancy-related fetal diseases (Charnock-Jones & Burton\textsuperscript{56}, 2000; Regnault\textsuperscript{93} et al. 2002; Torry\textsuperscript{94} et al. 2004). Some clinical studies revealed marginal insertion of the umbilical cord in about 42% cases of pregnancy-induced hypertension (Pretorius\textsuperscript{21} et al. 1996) and a correlation with low birth-weight babies (Rath\textsuperscript{25} et al. 2000). It was also observed that the mean number of infarcted areas and marginal insertion of umbilical cord was significantly higher in the mother hypertensive group in comparison with a control group (Majumdar S\textsuperscript{32} et al. 2005).

The hemodynamic analysis demonstrated that energy losses due to pressure gradients in the bifurcation region are smaller in the monopodial branching model than in the dichotomous model. This supports the fact that the monopodial pattern is more efficient for delivering blood long
distances, whereas the dichotomous pattern is efficient for perfusing large areas (Gordon et al. 2007).

The anthropometric data obtained from the 55 placental casts of this study were used to generate a typical architecture for the network of vessels that branch off the umbilical artery (Fig. 11). This typical geometry may be used for models of the vasculature for experimental or computational investigations of chorionic blood perfusion. The length of each generation was estimated to allow for the network to traverse the chorionic surface from cord insertion to placental perimeter. We assumed an average diameter of 200 mm to represent a typical healthy placenta. This model will allow development of additional studies of the efficacy of each network unit to provide homogeneous blood perfusion over the chorionic plate. In addition, the branching ratios comply with published data for monopodial and dichotomous branching segments (Onuma et al. 2001; Wang & Kraman, 2004).