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Short Contribution

The human placenta becomes haemochorial at the 13th week of pregnancy

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ABSTRACT Histological specimens of recent implantation sites are the basis of our current concept on human embryo implantation and placental development. In the Carnegie Collection maternal red blood cells were detected early in the primitive intervillous space (10th-12th day after conception). These cells were localized to the trophoblastic lacunae and originated from distended peripheral maternal sinusoids (Kaufmann, 1981). The classical theory states that progressively more and more maternal vessels are tapped. A true maternal blood flow is established around the 29th day. Dynamic investigations of human placental development in vivo are scarce and hampered by ethical considerations and the absolute requirement to refrain from using non aggressive and potentially harmful techniques. Despite these limitations such studies provide new insights that surprisingly contradict our previously and seemingly definitely established knowledge of the early phases of placental vascularization, and lead us to conclude that there is an absence of maternal blood circulation in the intervillous placental space (IVS) during the 12 first weeks of human pregnancy.

KEY WORDS: *haemochorial placenta, embryo implantation, placental vascularization*

Success of embryo implantation and pregnancy outcome in the human depends on formation of haemochorial placenta. The process requires a remarkable series of temporally and spatially regulated events involving highly specialized fetal and maternal cells (for a review, see Aplin, 1991).

The human placenta is an invasive organ analogous to a locally invasive tumor. It begins when the blastocyst attaches to the endometrial epithelium, penetrates its basement membrane and establishes contact with the underlying stroma (Schlafke and Enders, 1975; Denker, 1990). The external trophoblast layer of the blastocyst considerably proliferates and invades the maternal endometrium from the 7th day after fertilization. Soon thereafter, mononuclear cytotrophoblasts and multinuclear syncytiotrophoblasts are found mingled with maternal decidual cells throughout the site of implantation termed placental bed.

Groups of cytotrophoblasts migrate through the decidua, invade the walls of the spiral arterioles, replace the endothelial cells, disrupt the internal elastic lamina and the smooth muscle cells of the medial layer throughout the decidual segments of the arteries as far as myometrium (Boyd and Hamilton, 1970; Ramsey *et al.*, 1976; Foidart *et al.*, 1983; Hustin *et al.*, 1983a,b, 1984; Foidart and Lambotte, 1984; Schaaps *et al.*, 1989, 1990).

The wide openings and dilatation of these arteries result in the establishment of the human haemochorial placenta in which fetal chorionic villi are constantly bathed in maternal blood (Lala and Graham, 1990; Aplin, 1991).

Our knowledge of sequences of events is based mostly on the static information generated from sections of human implantation sites (Brosens, 1988) or from radioangiography and histological inspection of monkey specimens (Ramsey and Donner, 1988).

Data on the cellular molecular mechanisms that control placentation are starting to accumulate rapidly. The cell-cell and cell-matrix interactions and the cytokines that precisely regulate trophoblast proliferation, differentiation and invasion are extensively studied in *vivo*, in animal models and *in vitro* (Foidart *et al.*, 1990; Emonard *et al.*, 1990; Lala and Graham, 1990).

The translucent fluid that fills the intervillous chamber closely resembles plasma. Its flow rate is important. The burst of maternal blood at the 13th week of pregnancy provides a means of evaluating the rate of elimination of the maternal erythrocytes by the circulating plasma. As observed by cinematography the maternal blood emerges in a translucent liquid with an intense flow rate. From the 14th week the IVS is filled with maternal blood and the villi are no longer distinguishable.

Our observations raise several questions:

1. How can the fuel and oxygen sypply to the embryo be adequate?
2. Is it conceivable that the oxygen supply to the fetus is dependent on the partial pressure of oxygen dissolved in plasma ?

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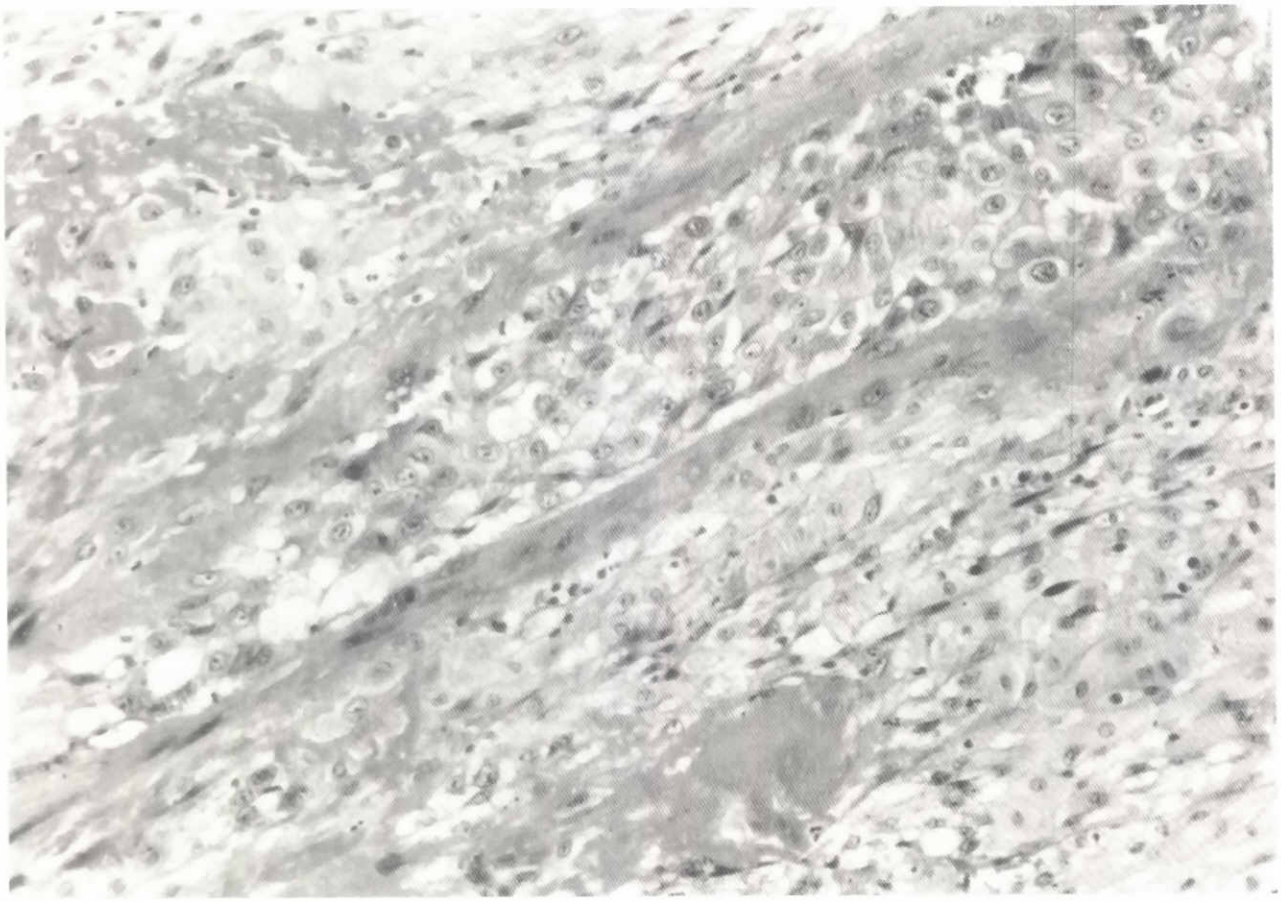

Fig. 1. Arterial migration of trophoblast cells with plug formation in a human uteroplacental artery - 12th week (x 40).

According to our view the physiology of placentation is divided into two phases. The first one lasts for 13 weeks. During this period of implantation, organogenesis and placental development, no haemochorial placentation is effective. The maternal plasma percolates between the trophoblastic plugs that fill the lumen of the uteroplacental arteries.

The events that encompass vertebrate phylogeny occur without maternal blood flow, probably under reduced oxygen-tension conditions. Such conditions closely resemble those occurring in fish and frog embryos that have external gills (i.e., the early villus placenta). The second period would encompass the last two thirds of pregnancy. They are characteristic of mammalian evolution. The increased fetal growth requires an active maternal blood flow in the IVS. Additional studies are of course mandatory to further strenghten or invalidate our challenging hypothesis.

Experimental Procedures

Echographic studies

Technological advances in ultrasound diagnosis have allowed internal contact examinations and color Doppler investigations. The content of the pregnant uterus is easily examined with a small linear 7 MHz probe inserted in the fundus of the vagina (Schaaps and Hustin, 1988; Schaaps *et al.*, 1990).

The gestational sac can be visualized from 4.5 weeks of amenorrhea on. Cardiac activity is detected when the heart begins to pulse (23rd day post-conception). The utero-trophoblastic border is also easily observed.

In several prospective studies, 409 patients underwent a vaginal ultrasonic inspection between the 6th and 14th week of pregnancy.

Moving echoes and color dots generated by the Doppler were visualized in the maternal vessels that surround the uterine border of the gestational sac but never in the thickness of the trophoblastic ring up to the 12th week of pregnancy. These uterine vessels were evident approximately 1 cm from the trophoblast ring. The moving-echoes were usually synchronous to the maternal heart rate. As pregnancy advanced, the vascular network became more conspicuous and was demonstrated in close vicinity to the trophoblastic shell from the 12th-13th week on. However, uterine vessels did not reach the placenta. No particulate movement could be detected in the trophoblastic area (Hustin and Schaaps, 1987; Hustin *et al.*, 1988; Schaaps and Hustin, 1988).

Video-camera recording in the intervillous chamber during early pregnancy

Chorionic villi sampling (CVS) is usually performed by transcervical insertion of a catheter, needle, or biopsy under echographic guidance. These samplings are usually bloodless (for a review, see Pescia and Nguyen The, 1986; Hustin and Schaaps, 1987). Using a chorionoscope or a small hysteroscope (2.5 mm diameter), we directly visualized the intervillous chamber after intrauterine insertion under echoguidance in 25 pregnant women. The manipulation is painless and allows a direct vision of free floating villi in the IVS. The villi are revealed as white structures resembling

the tentacles of a sea anemone bathing in a translucent fluid. No maternal blood is detected in the intervillous space up to the 12th week of pregnancy. The fetal circulation is easily observed in the stem villi.

From the 13th week, pulsatile maternal blood flow emerged in the intervillous space and bulged into the translucent fluid filling the IVS. Video recording clearly indicated that these spurts of blood arose from the maternal arteries and were synchronous to the maternal heart rate (a copy of these video tapes can be obtained from the authors upon request.

Morphologic studies

Since disruption of the IVS with curettage is inevitable, we focused our histological inspection on the implantation sites and the uteroplacental arteries of hysterectomy specimens taken from pregnant patients with cervical carcinomas.

The course of the spiral arteries was described from serial sections of selected blocks (Hustin and Schaaps, 1987). It demonstrated the presence of trophoblastic plugs occluding the uteroplacental arteries. Such obliteration of the maternal arterial connections to the placenta persisted up to the 12th week of pregnancy. These plugs extended toward the decidual and myometrial segments of the uteroplacental arteries. They have also been reported by others (Boyd and Hamilton, 1970; Ramsey and Donner, 1988) (Fig. 1).

Studies of X-ray films from one pregnant uterus injected through the uterine artery with barium sulfate confirmed the absence of radio-opacity in the IVS. Arteries were abundant in the insertion sites but were occluded. Histological examination showed that they were distended with a loosening of the trophoblastic plugs intermingled with the endothelial layer.

Physiological studies

The absence of maternal blood flow during the first 12 weeks of pregnancy raises the important question of oxygen supply to the embryo. We confirmed by culturing murine embryos (days 8-9), the possibility of observing the persistence of embryo development in the absence of maternal circulation. New *et al.* (1970) previously showed the survival of post-implantation murine embryo during organogenesis (24-48 h in culture). Using this methodology, we confirmed the possibility of maintaining well-differentiated embryos for 48 h in culture provided that the medium is regularly oxygenated during the rotatory culture conditions.

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