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ORIGINAL ARTICLE

Vascular system of human fetal pancreas demonstrated by corrosion casting and scanning electron microscopy

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Abstract Vascular architecture of the human pancreas was investigated by corrosion casting combined with scanning electron microscopy in fetuses aged 20 and 25 gestational weeks. The general pattern of the microvascular system was similar to that of the postnatal pancreas, with an evident insulo-acinar portal system and with three types of capillary networks: capillaries of exocrine lobules, islet capillaries and periductal capillaries around large ducts located in the interlobular septa. All these capillary networks were supplied by arteriolar branches of the interlobular arteries. As compared with the postnatal pancreas, capillaries of exocrine lobules formed denser meshworks, had a more sinusoidal character and revealed morphological features indicative of angiogenesis (blind capillary sprouts). The number of efferent (portal) capillaries per islet was lower and the predominant pattern of islet vasculature was top to bottom rather than inner to outer, as observed in adults. These results show that in the second trimester the human pancreatic vascular architecture is almost completely developed and requires only minor remodeling to be fully functional in the postnatal period.

Keywords Blood vessels · Corrosion casting · Human fetus · Pancreas · SEM

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Introduction

The microvascular architecture of pancreas has a peculiar functional significance, since it integrates the exocrine and endocrine compartments of the organ. Early studies employed dye injection techniques, microangiography or serial sections to investigate the vascular system of the human pancreas (Beck and Berg 1931; Wharton 1932; Thiel 1954; Kivisaari 1979; Yaginuma et al. 1986). For the last 3 decades, vascular corrosion casting combined with scanning electron microscopy (SEM) has been the preferred technique to study morphology of the microvascular systems, since it offers high resolution and attractive quasi-three-dimensional images of the studied vascular networks (Lametschwandtner et al. 1990). With the use of this technique, the pancreatic microvasculature was extensively investigated in laboratory animals (Ohtani et al. 1986; Aharinejad et al. 1993; Murakami et al. 1993; Ohtani and Wang 1997), but only a few studies concerned the human pancreas (Murakami et al. 1992, 1994, 1997; Petruzzo et al. 1997), and they were performed on autopsy material collected from adult individuals. Development of the human pancreatic vasculature during the fetal period has not yet been studied by corrosion casting, and in the present study that technique was used to reveal the vascular architecture of pancreas in the second/third trimester.

Materials and methods

The investigations were carried out on two female fetuses, aged 20 and 25 gestational weeks, belonging to the material collected for vascular corrosion cast studies in the 1987–1994 period in accordance with institutional requirements for the use of human material. The fetuses were obtained after spontaneous abortions from the Obstetric Clinic of the Jagiellonian University Medical College. The abortions were due to maternal disorders, and no developmental malformations or vascular anomalies were found in the fetuses upon macroscopic inspection.

After abortion, the thorax of the fetus was opened to expose the heart and large vessels. The heart apex was cut off, and a cannula was inserted via the left ventricle into the aorta and fixed by ligation at the level of the ascending part. The vascular system of the fetus was subsequently perfused manually by a sequence of solutions, with the outflow occurring via the umbilical vessels and additionally incised posterior tibial veins.

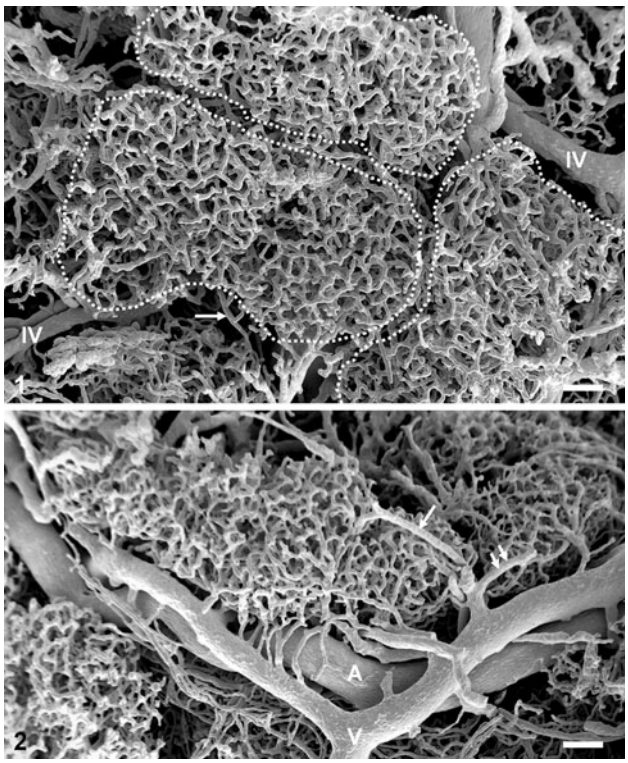
The fetuses were perfused with prewarmed (37°C) heparinized saline (12.5 IU ml^{-1}) until the outflowing saline was clear, devoid of blood or clots. Next, perfusion fixation was carried out with modified Karnovsky's fixative containing 0.66% freshly prepared paraformaldehyde, 0.08% glutaraldehyde and 0.025% lidocain (Lignocain, Polfa) in 0.2 M cacodylate buffer, pH 7.4, at 37°C. Finally, fetuses were perfused with low viscosity Mercox CL-2R resin (Vilene Comp., Tokyo, Japan) containing 0.2 g MA polymerization initiator per 10 ml of the resin. Following the perfusion, the fetuses were kept overnight in a water bath at 55°C in order to facilitate resin polymerization.

After resin curing, the pancreas together with the adjacent duodenum and spleen was dissected out, washed several times with distilled water and macerated at 37°C in 10% potassium hydroxide for 30 days, with daily changes of the solution and alternating washes in distilled water. The resulting vascular casts were carefully and thoroughly cleaned in 5% trichloroacetic acid, followed by washing in distilled water for a few days. The casts were then freeze-dried, mounted onto specially prepared large specimen holders using colloidal silver and "conductive bridges" (Lametschwandtner et al. 1980), coated with gold and examined in a Jeol JSM 35-CF scanning electron microscope at 20–25 kV.

After preliminary examination and photographic documentation of the extrinsic vascular system of the pancreas, the casts were removed from the holders, embedded at 55°C in polyethylene glycol (PEG), cooled to room temperature to solidify PEG and sectioned to reveal the intrinsic vessels. The sectioned fragments were washed with stirred distilled water to remove PEG, freeze-dried, remounted, again coated with gold and used for the next series of scanning electron microscopic observations.

Results

The general vascular architecture of the pancreas in the investigated fetal period was similar, and no substantial differences were observed between the 20- and 25-week fetus. The division of the gland into lobules was already marked. The narrowest interlobular septa usually did not contain interlobular blood vessels (Fig. 1). The larger septa showed the presence of interlobular artery and vein running in parallel (Fig. 2) and sometimes accompanied by capillary plexus surrounding the interlobular pancreatic duct. Such plexuses were observed mostly in the vicinity of the largest interlobular vessels (Fig. 3) and only occasionally in narrower septa containing smaller arterial

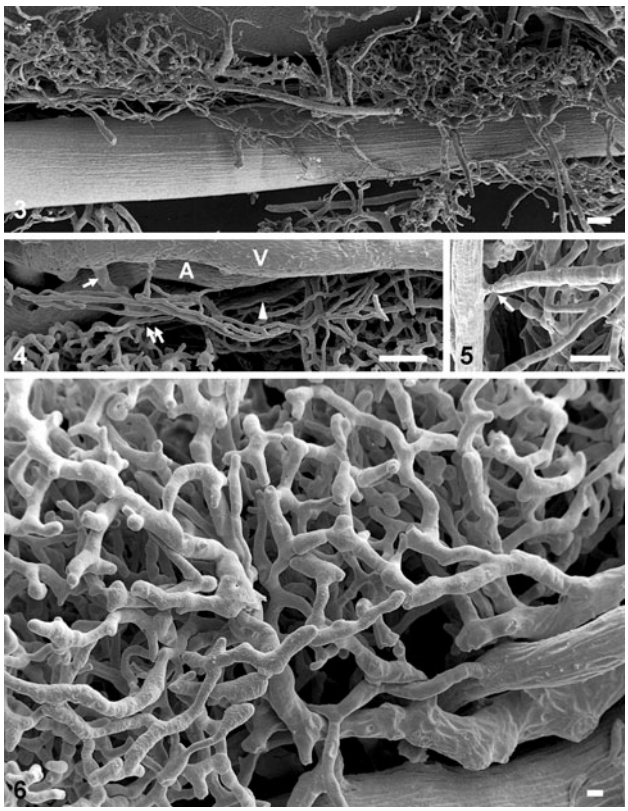


Figs. 1–2 **1** Three adjacent exocrine lobules (demarcated by *dotted lines*) separated by narrow septa and interconnected by numerous capillary vessels. Interlobular vessels (*IV*) run in wider septa; lobules separated by such septum show very few capillary interconnections (*arrow*). A 25-week fetus. *Bar* 100 µm. **2** Interlobular vessels (*A* artery, *V* vein) and the capillary networks of adjacent lobules. Arteriole supplying one of the lobules and venule draining it are indicated by a *single* and *double arrow*, respectively. Note parallel capillaries leaving the periphery of the lobule and draining directly into the interlobular vein. There are no direct capillary connections between the lobules flanking the interlobular vessels. A 25-week fetus. *Bar* 100 µm

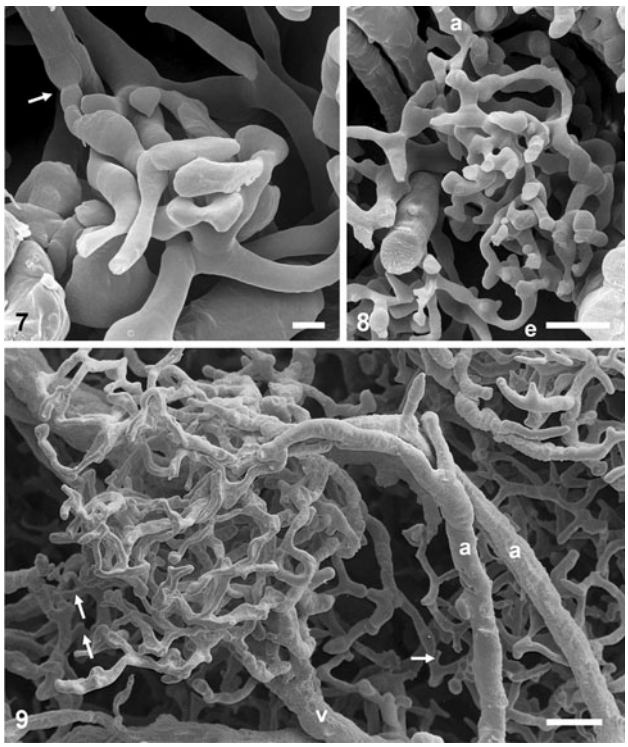
and venous branches. In the latter cases, the periductal plexus was formed by a strand of elongated capillaries interconnected by short transverse connections in a ladder-like fashion (Fig. 4). The plexuses associated with ducts were mostly supplied by short afferent arterioles originating from interlobular arteries and drained by branches of interlobular veins, but communication of the periductal plexus with a capillary network of the lobules was also observed (Fig. 4).

The exocrine lobules, 350–800 μm in size, were supplied by 1–3 short arterioles, 25–50 μm in diameter, branches of the interlobular arteries (Fig. 2). At the site of origin, the intralobular arteriole casts often revealed distinct annular constrictions suggesting the presence of some kind of sphincter. Similar albeit shallower constrictions caused by contracted vascular smooth muscle cells were also observed along the arteriole (Fig. 5). Shortly after entering the lobule, the arterioles branched into a very dense capillary plexus with irregular meshes 20–40 μm in diameter. The capillaries had an average diameter of 8–12 um, although areas with thinner (5 μm) and thicker (up to 18 μm) capillaries were also encountered. They were characterized by uneven contours with thicker and thinner portions, and occasionally showed structures most likely corresponding to blind capillary sprouts (Fig. 6). The capillary network of the lobule was drained by intralobular venules, up to 50 μm in diameter, usually located close to the lobule periphery, which joined the interlobular veins (Fig. 2). Occasionally, very short afferent and efferent vessels of capillary size connected the periphery of the lobule with interlobular artery or vein (Fig. 2). Direct capillary connections between adjacent lobules were observed frequently when the interlobular septa were narrow (Fig. 1), but very rarely did such connections penetrate wide septa containing interlobular vessels (Fig. 2).

In the corrosion casts, the location of the pancreatic islets inside the exocrine lobules was difficult to discriminate against the background of very dense capillary network of the lobules. Microvascular systems of the islets could be identified as glomerular structures ranging from 100 to 250 μm in diameter, with slightly thicker capillaries often showing irregular, sinusoidal contours. The capillaries of smallest islets were integrated with the surrounding capillary system of the lobule (Fig. 7). Larger islets were supplied by 1–2 afferent ramifications of the intralobular arterioles, which mostly branched on the periphery of the islet and more rarely penetrated into it. The vessels supplying the islets often showed sphincter-like constrictions (Fig. 7). The efferent vessels of capillary size (usually 2–5 per islet) joined the capillary plexus of the lobule (Figs. 7, 8). In larger islets located peripherally in the lobule, apart from efferent capillaries, a single efferent vessel of a larger diameter (a venule) was occasionally observed to leave the islet and drain to the interlobular vein (Fig. 9). In the majority of cases, the afferent and efferent vessels were located at the opposite sides of the islet (Figs. 7, 8, 9). On very rare occasions, glomerular capillary plexuses were observed in wide interlobular septa, suggesting the presence of extralobular islets. They had large size (350–420 μm) and a different microvascular pattern. Such plexuses had their own afferent arterioles (2–3) originating from interlobular arteries, were composed of thin, more regular, and thick, sinusoidal capillaries, and were drained by efferent venules joining interlobular veins. There were no connections between the capillaries of these extralobular plexuses and the capillary network of the lobules (Fig. 10).



Figs. 3–6 **3** Two large interlobular vessels accompanied by periductal plexus. A 25-week fetus. *Bar* 100 μm. **4** Slender periductal plexus supplied by arteriolar branch (*arrowhead*) of interlobular artery and drained by a venule emptying into interlobular vein (*arrow*). Capillary connection between the plexus and the adjacent exocrine lobule is indicated by a *double arrow*. *A* artery, *V* vein. A 25-week fetus. *Bar* 100 μm. **5** Intralobular arteriole branching off the interlobular artery. Note a sphincter-like deep annular constriction (*arrow*) at the site of arteriole origin and shallower constrictions probably related to arteriolar smooth muscle cells. A 25-week fetus. *Bar* 50 μm. **6** Capillary network of the exocrine lobule showing very high density of the vessels and numerous blind capillary sprouts. The capillaries have uneven contours with thinner and thicker portions. A 20-week fetus. *Bar* 10 μm



Figs. 7–9 **7** Capillary plexus of a small islet integrated with lobular capillaries. The afferent vessel shows annular, sphincter-like constrictions (*arrow*); the efferent vessels leave the plexus at the opposite pole. A 20-week fetus. *Bar* 10 µm. **8** Capillary plexus of a larger islet showing single afferent (*a*) and efferent (*e*) vessels with top-to-bottom pattern. A 20-week fetus. *Bar* 50 µm. **9** Capillary plexus of a large islet located near the periphery of the lobule with two afferent arterioles (*a*) supplying the plexus at its upper pole and with efferent venule (*v*) at the bottom. Efferent capillaries joining the capillaries of the lobule are indicated by *arrows*. A 25-week fetus. *Bar* 50 µm

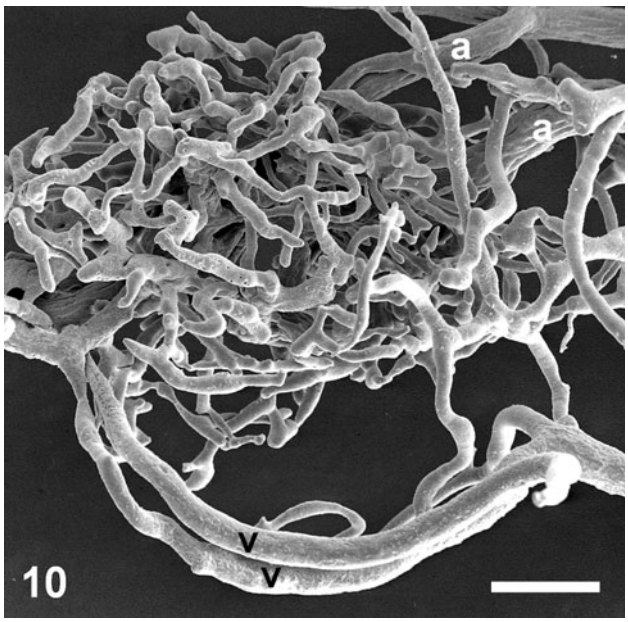


Fig. 10 Glomerular capillary plexus located in the extralobular space supplied by two arteriolar branches (*a*) of the interlobular artery and drained by two venules (*v*). The plexus shows no capillary connections to the exocrine lobules. *Bar* 100 µm

Discussion

The general vascular pattern of the fetal pancreas in the 2nd/3rd trimester was similar to that described by Murakami et al. (1992, 1994) for adult individuals. The observed differences concerned details of the system rather than its architecture. As compared with casts of pancreatic exocrine lobules in the postnatal period, the density of capillaries observed in our material was distinctly higher. The diameter of meshes in the capillary network of fetal pancreas mostly ranged from 20 to 40 µm, whereas the respective range in adult pancreas was 30–70 µm, corresponding to the size of exocrine acini. This difference seems to reflect the developing histological organization of fetal pancreas, since acini arise by differentiation of duct cells during the 14- to 20-week stage, first as irregular periductal proliferation, and they acquire their final form and size with lumen and centroacinar cells after the 6th month of pregnancy (Liu and Potter 1962; Laitio et al. 1974). During that process, the capillary network has to be remodeled to adopt the "growing" acini, and this remodeling might cause the increase in the size of meshes and, secondarily, decrease in vascular density.

The appearance of capillaries in our casts also differed from that presented by Murakami et al. (1992, 1994): they had larger diameter and irregular contours with constrictions and nodular thickenings, whereas in the postnatal pancreas capillaries of the exocrine compartment were thin and regular. In mouse and rat pancreas, the undulating and bulged capillary casts were demonstrated to correspond to fenestrated capillaries (Aharinejad and Böck 1994; Aharinejad et al. 1997). Combination of corrosion casting and in vivo microscopy revealed that capillary constrictions observed in the cast specimens are due to local contraction of endothelial cells, which contribute to blood flow regulation in the pancreatic microvasculature (Aharinejad et al. 1993; 1997; MacDonald et al. 1995). It is, however, uncertain whether the functional significance of those morphological capillary features demonstrated in laboratory animals can apply to human fetal pancreas, especially since they are absent from the microvasculature of "adult" gland (Murakami et al. 1992). Moreover, a "sinusoidal" character of capillaries seems to be characteristic of fetal microcirculation, as similar capillary morphology was observed in corrosion casts of other fetal organs (Skladzien et al. 1995; Gorczyca et al. 1998, 1999).

In the cast arterioles, at the sites where they branch off, we occasionally observed relatively deep annular constrictions, most likely corresponding to smooth muscle

sphincters regulating blood supply to the local capillary beds. In animal pancreas, such sphincters were also observed—but in veins (Aharinejad et al. 1997)—which also stresses the differences between the animal and human fetal pancreatic microcirculatory systems.

The fetal capillary networks of both exocrine acini and endocrine islets showed relatively numerous blind, narrow capillary sprouts, which might be attributed to ongoing angiogenesis (although some of these profiles could have been the result of incomplete perfusion by resin).

During the fetal period investigated in this study, although islets can still be in contact with smaller ducts (Watanabe et al. 1999), they form separate entities, and the islet cells undergo internal reorganization, first forming mantled islets with beta cells being surrounded by non-beta cells, which later enter inside the islet (Hahn von Dorsche et al. 1988). The microvasculature of the islet is also developed, since capillary vessels penetrate the islets as early as 14 w.p.c. (Piper et al. 2004).

Our observations have confirmed the occurrence of the insulo-acinar portal system (Ohtani and Fujita 1981; Murakami et al. 1992) in the human fetal pancreas—the efferent vessels of the pancreatic islets communicated with the capillaries of exocrine lobules and only rarely (in islets located peripherally in the lobule) also drained directly to larger veins. The predominant microvascular patterns of the fetal pancreatic islets, however, differed from that described in the pancreata of adult individuals by Murakami et al. (1992), who found in most islets afferent vessels entering deep into the islet and numerous (from a few up to 30) efferent capillary vessels leaving the periphery of the islet and joining the capillary network of the lobule. This corresponds to the so-called inner-to-outer microcirculation pattern of the islets (Nyman et al. 2008). In the fetal pancreas, the number of efferent vessels leaving a single islet was limited (mostly 2–5), and they usually left the islet on the opposite pole to that of afferent vessel(s) location (top-to-bottom pattern). Such a pattern was also observed, albeit rarely, in adult pancreas by Murakami et al. (1992). Since the latter authors presented convincing micrographs supporting the inner-to-outer pattern as the predominant microvessel architecture of the human islets, it seems justified to suggest that in the course of fetal development the blood vessels penetrating the developing islets first form the bipolar (top-to-bottom) network, which is later remodeled to acquire the inner-to-outer arrangement.

The microvascular architecture of the pancreatic islets is believed to have a considerable functional significance, since the order of perfusion determines the interactions between islet cell types. It has been postulated that in the majority of mouse islets beta cells forming the islet core are perfused first, and their secretory products have regulatory effects on other cell types located at the periphery of the islet (Nyman et al. 2008). However, recent studies have shown that the human islets have a different cytoarchitecture than mouse or rat islets in which the beta cell core and non-beta cell mantle are evident. In human islets, all cell types are rather randomly scattered throughout the islet and located along blood vessels without any particular order (Brissova et al. 2005; Cabrera et al. 2006). Hence, it cannot be excluded that in the human islets the microvascular pattern plays only a minor, if any, functional role, and the interactions between islet cells have predominantly a paracrine character.

The large glomerular capillary plexuses separated from the lobular capillary networks and located in the interlobular septa should be interpreted with caution. On one hand, Murakami et al. (1992) described them as extralobular islets, and this interpretation was cited in some papers and monographs published by other authors. On the other hand, the existence of extralobular islets in normal human pancreas is disputable, since to the best of the authors' knowledge such islets have not been demonstrated in routinely processed sections by either light or electron microscopy. Furthermore, in an immunocytochemical study on human pancreas, direct topographical association was never found between islet endocrine cells and epithelial cells of large (interlobular and main) ducts located in the interlobular septa (Zhao et al. 2008). Formation of extralobular islets by outgrowth and differentiation of excretory duct cells was observed in isolated and cultured pathological pancreatic explants (Hollande et al. 1976), and the authors interpreted that phenomenon as activation of embryonic developmental mechanisms in cultured mature tissue. At the stage of fetal development investigated in the present study, islets are already separated from the interlobular ducts and surrounded by the exocrine tissue. Hence, although persistence of some extralobular islets closely associated with large ducts cannot be excluded with certainty, it seems much more likely that extralobular glomerular plexuses represent capillary systems of relatively small clusters of exocrine acini located in the interlobular septa. Such "exocrine islets" in the interlobular connective tissue are sometimes observed in the pancreata of humans and animals.

In conclusion, the human pancreatic vascular architecture is almost completely developed by the 20th week of gestation and requires only minor remodeling to be fully functional in the postnatal period.

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