Dept. of Anatomy and Histology,
Fac. Vet. Med., Assiut University

MICROVASCULAR ARCHITECTURE OF THE MARE PLACENTA AT MID-GESTATION: A LIGHT AND SCANNING ELECTRON MICROSCOPICAL STUDY

(With 7 Figures)

By

M. M. M. ABD-ELNAEIM

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التراكيب الوعائية الدقيقة في مشيمة الفرس عند منتصف فترة الحمل: دراسة بالميكروسكوب الضوئي والماسح الإلكتروني

محمود محمد محمود عبد النعيم

أجريت هذه الدراسة على عدد (٦) من أنسجة الرحم والمشيمة في الأفراس عند نهاية الشهر السادس من فترة الحمل؛ لتوضيح شكل التراكيب الدموية في الوحدة الوظيفية للمشيمة (*Microplacentome*)، ولهذا فقد أعدت أنسجة الرحم والمشيمة لاستخدام الميكروسكوب الضوئي بالطرق المعروفة، أما قوالب الأوعية الدموية الدقيقة فقد أعدت عن طريق حقن خليط مكون من ميركوكس (٢٠) مللي، ميثيل ميثاكريلات (٥) مللي والعامل المساعد (٠,٥) جم، وقد تم حقن هذا الخليط عن طريق تفرعات الشرايين السُرية والرحمية، وكليهما للحصول على قوالب الأوعية الدموية لمشيمة الجنين والأم والقوالب المركبة بالتوالي. أثبتت الدراسة أن الفِلقات في الغشاء السلي-المنباري كانت كُروية ومنغمدة تماماً فيما يقابلها من تجاويف عميقة في اللُحيمات (*Microcaruncles*) في جدار الرحم لتكون وحدات وظيفية (*Microplacentomes*) ذات أقطار مختلفة تتراوح مابين (٢٤٠-٦٦٠) ميكرون. وقد أوضحت الدراسة أن كل فِلقة في الغشاء السلي-المنباري تتغذى عن طريق شريان فِلقي دقيق واحد حيث إن هذا الشريان يتفرع إلى (٤-٥) شرايين جذعية صغيرة؛ لتغذية الخملات الجذعية، ويقوم كل شريان جذعي بالانقسام إلى (٦-٨) شرايين مُتوسطية؛ لتغذية الخملات المقابلة. أما المدد الدموي للخملات النهائية فقد تكون من شبكة مُحكمة من الشَعيرات الدموية التي تمتد كل منها لتكون (٣-٥) عُروة شَعرية، وأن كل عُروة شَعرية تكونت من طرَف شَريانية وسطية دقيقة ذات قطر يبلغ حوالي (١٢) ميكرون، وأن الدم الوريدي يخرج عن طريق أطراف وريدية نهائية دقيقة ذات أقطار تبلغ حوالي (١٥) ميكرون. أن المدد الدموي الشرياني لكل لُحيمة دقيقة يأتي عن طريق (٢) من الشرايين اللُحيمية المستقيمة التي تمتد حتى فتحة اللُحيمية وعندها أو قبلها بقليل يتفرع كل شريان إلى (٢-٤) شرايين صغيرة ذات أقطار يبلغ متوسطها (٤٠) ميكرون. هذه الشرايين الصغيرة تسلك طريقاً قصيراً قبل التفرع أو تتفرع مباشرة إلى شَعيرات دموية كثيفة غير منتظمة وذات أقطار تصل إلى (٩) ميكرون عند فتحة اللُحيمية الأساسية. هذه الشَعيرات تمتد لتكون شبكة شَعيرية دموية تشبه خلايا عسل النحل عند جوانب وقاعدة اللُحيمية.

تزداد أقطار الشعيرات الدموية تدريجياً عند قاعدة كل لحيمية (١٢) ميكرون، وتمتد لتكون أطرافاً وريدية وأوردة لحيمية يتراوح متوسط أقطارها مابين (١٥-٦٠) ميكرون. تتجمع هذه الأوردة الصغيرة لتكون وريداً لحيمياً كبيراً يقوم بعملية نقل الدم من اللحيمية إلى الوريد الرحمي. أثبتت هذه الدراسة أيضاً، أن النظام الوعائي الدموي في مشيمة الفرس يعد معقداً إلى حد ما وخصوصاً في الخملات النهائية الجنينية وما يقابلها من لحيميات صغيرة جداً في مشيمة الأم، وهذه المنطقة تعد الأكثر نشاطاً في عملية التبادل بين الأم والجنين. وأن ترتيب الأوعية الدموية الدقيقة (شرايين وأوردة صغيرة جداً وشعيرات دموية) في هذه المنطقة يشير إلى أن سريان الدم يتبع نظام الاتجاة المعاكس، ويعد هذا النظام من أكثر الأنظمة فعالية في عملية التبادل التي تحدث في الحيوانات المستأنسة، وكونه يحدث بشكل مُركب في مشيمة الفرس فهذا يضيف إلية فعالية أكثر في عملية التبادل بين الأم والجنين.

SUMMARY

The present study was carried out on 6 utero-placental tissue specimens of pregnant mares at the end of the six month of gestation to clarify the blood vascular architecture of the microplacentomes. For light microscopy, perfusion fixation through the uterine and umbilical arteries was performed using 3 % glutaraldehyde in 0.1 M phosphate buffer. Semithin sections of about 1 µm thick were prepared and stained with 1% toluidine blue. The microvascular casts were obtained by injection of liquid plastic of three components (20 ml Mercox, 5 ml Methylmethacrylate and 0.5 g catalyst,) through branches of the umbilical arteries (fetal casts), uterine arteries (maternal casts) and through both (combined feto-maternal vascular casts). The microcotyledons were globular in shape, and completely enclosed in the corresponding microcaruncles, constituting microplacentomes of variable diameters (240-660 µm). The microcotyledon consists of variable numbers of stem villi (4-5) and each villus is divided into 6-8 intermediate villi. The latter is subdivided into 4-5 terminal villi, which penetrated deeply into the endometrial tissue to anchor with the corresponding maternal crypts. The microcotyledon was vascularised through a single microcotyledonary artery and drained by 2-3 microcotyledonary veins. The artery followed a short straight course before it is divided into 4-5 stem arteries. The stem artery is subdivided into 6-8 smaller arterial branches according to the number of the intermediate villi. The stem veins were smaller and located peripheral to the artery, more than one stem vein were converged onto microcotyledonary vein which joined the allantochorionic veins. For each intermediate villus, one artery of small diameter or arteriole (15-25 µm) could be observed giving off 4-5 arterioles to vascularise the terminal villi. The venules were originated at different levels from the

capillary complex and located peripheral to the artery. The vasculatur of the terminal villi was formed by tightly arranged capillaries, and each terminal villous capillary ended by 3-5 capillary loops. The latter is consisted of centrally located arterial capillary limb (12 μm) average diameter, and drained by 1-4 venous capillary limbs (15 μm) average diameter. The microcaruncle was generally supplied by two straight arteries that ran in a materno-fetal direction to the orifice (primary crypt) and at this location each artery is ramified into 2-4 arterioles (40 μm) average diameter. These arterioles ran a short course and/or directly ramified into capillaries constituting the capillary networks of the orifice, side and base of microcaruncle. At the orifice maternal capillaries were relatively dense and irregularly oriented but they were organized in a honey-comb like fashion at the side and base of the microcarucle. These capillaries appeared rounded to oval in cross section and larger (9 μm average diameter) than the fetal ones. However, they were flattened and enlarged (12 μm) average diameter, before converging onto venules. The venous drainage of the microcaruncle occurred by a large number of venous capillary limbs and venules (15-60 μm) average diameter. These venules were originated from the capillary complex of microcaruncle at its base and then converged to constitute a large microcaruncular vein. The results indicated that the fetal and maternal placental vessel systems are complicated particularly at the most working part (terminal villi) with blood flow which occurs generally in the opposite directions. This creating a "composed" countercurrent feto-maternal blood flow interrelationship, which is the most efficient system for exchange in the placentae of domestic animals.

Key words: *Horse, Placenta, Microplacentome, Vascularization, Casts, Light and scanning electron microscopy*

INTRODUCTION

The placenta is an organ with a limited life span, and is considered to be the only channel for transport of nutrients from the mother to the conceptus. The demands of this structure increase exponentially with the progress of pregnancy, and are accompanied by a wide variety of structural modifications (Wooding and Flint 1994). Within the different types of placentae, such structural modifications depend on factors including placental shape, materno-fetal

interdigitation, the layers comprising the interhaemal membrane, the degree of trophoblast invasiveness and materno-fetal blood flow interrelationships (Leiser and Kaufmann, 1994). In the horse, the placenta is diffuse, complete, microcotyledonary and epitheliochorial (Amoroso, 1952; Tsutsumi, 1962; Steven and Samuel, 1975; Samuel *et al.*, 1974, 1975, 1976; Steven 1982; Dantzer and Leiser, 1992 and Leiser and Kaufmann, 1994), with previous study suggesting that the feto-maternal blood flow interrelationship is multivillous (Leiser and Kaufmann, 1994).

One of the most important placental structural modifications involves the fetal and maternal vasculature, the volume of which depends particularly on the size of the microcotyledon. The latter increases gradually with the progress of pregnancy (Samuel *et al.*, 1974), and it reaches 1-2 mm average diameter at term (Wooding *et al.*, 2000). However, placental transport depends mainly on the nature of the interhaemal barrier which is epitheliochorial in the horse (Björkman 1968 and Silver *et al.*, 1973), consisting of the walls of the fetal and maternal blood vessel systems, separated by the uterine and chorionic epithelia. Despite the importance of the feto-maternal blood vascular systems; there is lack of information concerning the microvascular architecture of the horse placenta. Therefore, the present study aims to elucidate the microvasculature of both the fetal and maternal components of the placenta at mid-gestation. For this purpose, the histology of perfusion-fixed specimens have been compared with corresponding microvascular corrosion casts.

MATERIAL AND METHODS

Six utero-placental tissue specimens from pregnant mares at the end of the 6^{th} month of gestation were used to study the vascular architecture of the horse microplacentome. These materials were obtained from the Veterinary Hospital of Sao Paulo University.

For light microscopy, perfusion fixation through the uterine and umbilical arteries was performed using 3 % glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, followed by immersion fixation for 2 h in the same fixative. Then the specimens were washed several times in the same buffer. After osmication in 1 % OsO_4 for 2 h at the room temperature, the specimens were washed in the same buffer, then dehydrated in graded ethanol series and embedded in Araldite 502 (Luft, 1961). Semithin sections, 1 μm thick, were prepared on a Reichert OM

UII ultramicrotome and stained with 1% toluidine blue in 1% borax. The sections were examined and photographed using a Zeiss Axiophot light microscope.

Vascular casts were prepared from all placentae and at different areas of the allantochorionic sac. Small pieces, approximately 10 X 10 cm in size taken from separate and combined fetal and maternal placental tissues. The casts were obtained by injection of a liquid plastic through a branch of the umbilical artery (fetal casts), uterine arteries (maternal casts) and through both (combined feto-maternal vascular casts). The liquid plastic was freshly prepared from 0.5 g catalyst, 20 ml Mercox (Vilene, Tokyo, Japan) and 5 ml methylmethacrylate (Fluka, Neu-Ulm, Germany). The mixture was instilled at low constant hand pressure until the venous outflow consisted of pure plastic. After complete polymerisation of the plastic (30-60 min), the tissues were stored in a water bath at 60°C overnight. Corrosion was conducted by alternate immersion in 20-30 % potassium hydroxide at 60°C and running tap water (Leiser and Kohler, 1983). For mounting, the casts were immersed in distilled water and cut into small pieces by using scissors or frozen at –5°C in gelatine and fractured by using liquid nitrogen. Suitable air-dried specimens were selected by stereomicroscopy, mounted on aluminium stubs, sputter-coated with gold, and examined and photographed in a Zeiss scanning electron microscope (DSM 940).

Estimation of the average diameter of the microplacentomes and the external diameter of blood vessels and capillaries were done by using the image analysis programme AnalySIS 2.0 (SIS, Münster, Germany).

The United Medical Dictionary (Anouti *et al.*, 1983) was used in the translation from English to Arabic for writing the Arabic summary.

RESULTS

The fetal microcotyledons were numerous and completely covered the entire surface of the allantochorion. They were globular in shape with a narrow base and wide apex. These microcotyledons were completely enclosed in the corresponding maternal microcaruncles to constitute the functional unit of the horse placenta, the microplacentomes (Fig. 1a). The size of the microplacentomes was variable, ranging from 240 to 660 µm in diameter. The fetal microcotyledon consisted of variable numbers of stem villi (4-5) that were continued in a feto-maternal direction, and each one splited at

different levels into 6-8 intermediate villi (Fig. 1a, 1b). The latter was subdivided into 4-5 terminal villi. The terminal villi were penetrated deeply into the endometrial tissues to anchor with the corresponding maternal crypts of microcaruncles (Figs. 1a, 1b, 3a).

The allantochorionic arteries and veins were randomly distributed in the allantochorion and ramified into branches that ran along the fetal surface of the placenta to give the microcotyledonary arteries and veins (Figs. 1a, 2). The microcotyledonary artery assumed a short straight course in the stalk before it was divided into stem arteries at the base of a microcotyledon to supply the stem villi. The microcotyledonary veins (2-3) were small and seen peripheral to the artery (Fig. 2).

The vasculature of a stem villus was derived from one stem artery which arose from the microcotyledonary artery. The stem artery usually ran straight in the centre of the stem villus (Figs. 1b, 3a, 3b) and at different levels it was divided into 6-8 smaller arterial branches or arterioles (Figs. 1b, 3a, 3b). From the stem villus, more than one stem veins were collected to constitute the microcotyledonary veins which left the microcotyledon at its base to join the allantochorionic veins (Figs. 1b, 2, 3b).

For each intermediate villus, one central artery of very small diameter (15-25 μm) could be observed (Figs. 1b, 3a, 3b, 3c). The artery of the intermediate villus gave off 4-5 arterioles at an acute angle and at the same level, which extended to vascularise the terminal villi (Fig. 3a, 3b, 3c). The venules were originated at different levels from the capillary complex of the intermediate villus and located peripheral to the artery (Fig. 3a, 3c, 3d).

The vascular architecture of the terminal villi was formed by tightly arranged capillaries (Figs. 3b, 3d, 4a). Each terminal villous capillary extended by 3-5 capillary loops that formed sinusoidal dilated capillaries at the top (Figs. 3d, 4a, 4b). The capillary loop was consisted of a centrally located arterial capillary limb, 12 μm average diameter, and was drained by 1-4 venous capillary limbs, 15 μm average diameter (Fig. 4b, 4c, 4d). The venous capillary limbs, however, were rarely seen in a central location and usually originated a short distance from the top of the terminal villi (Fig. 4d). The fetal capillaries were generally smaller, of variable diameters, 8 μm average, and highly convoluted in comparison with the maternal ones (Figs. 3d, 4a). The shape of the capillaries appeared round to oval in cross sections (Fig. 4c, 4d), with

rough surfaces and circular impressions of endothelial cell nuclei on the vascular casts (Fig. 4e).

On the maternal side, the vascular architecture of the microcaruncles was relatively simple compared to that of the microcotyledons. Thus, the vascular frame of the microcaruncles was formed by the microcaruncular arteries which were originated from the endometrial vessels to form globular capillary systems in deeply-ramified maternal crypts (primary and secondry) (Fig. 5a, 5b, 5c).

Each microcaruncle was supplied by one straight microcaruncular artery on each side that ran to reach the top of microcaruncle at its fetal side (Fig. 5c). Shortly before the top of the microcaruncle each artery was ramified into 2-4 arteriolar branches, 40 μm in average diameter, that ran a very short course and/or directly ramified into capillaries to constitute the network of vessels found in the orifice "primary crypt", at the side, and at the base of microcaruncle (Figs. 5c, 6a, 6b). At the primary crypt or orifice of the microcaruncle, secondary crypts, (normally occupied by the fetal stem villi) were noticed. Here, maternal capillaries were relatively dense and irregularly oriented (Fig. 6b). At the side and base of the microcarucle the maternal capillaries were organized in a honey-comb like fashion (Figs. 5c, 6e).

The venous drainage of each microcaruncle was occurred through large numbers of venous capillary limbs and venules (15-60 μm). These venules were originated from the capillary complex of the microcaruncle at its base (Fig. 6c, 6d, 6e) and then converged to constitute a large microcaruncular vein (Figs. 5c, 6e).

Maternal capillaries of microcaruncles were oval to round in cross sections (Fig. 4d) and larger, 9 μm average diameter, than the fetal ones. At the base of microcaruncle, these capillaries were flattened and greatly enlarged to an average diameter of 12 μm, before converging onto maternal venules (Fig. 6c, 6d, 6e).

DISCUSSION

This study of the horse placenta at mid-gestation was limited by the materials obtained, however, this investigated period had the advantage that the placenta was not overly complicated in structure, despite the fact that the microcotyledons are fully formed and the vascular system was already established by day 150 of gestation (Samuel *et al.*, 1975).

The terms microcaruncle, microcotyledon and microplacentomes were used in the horse placenta by several investigators (Samuel *et al.*, 1974, 1975, 1976; Steven and Samuel, 1975; Steven, 1982; Dantzer and Leiser, 1992 and Leiser and Kaufmann, 1994). This was according to the similarity of the branching pattern of the fetal villi and the corresponding maternal crypts into several orders in the horse and ruminants placenta. In ruminants the caruncles are projections on the endometrium and are present also in the non-pregnant animals (Amoroso, 1952; Hafez, 1954 and Abdel-Rauf and Badawi, 1966) but in the horse the microcaruncles are deep ramified troughs or depressions in the endometrium of the pregnant animals and are not found in the non-pregnant ones.

Microcotyledonary and microcaruncular arteries, veins, arterioles, venules and capillaries could be clearly distinguished either histologically or from the vascular corrosion casts. Histologically, the arteries and arterioles were usually of smaller diameters than the veins and venules, round in cross sections, with relatively thick walls that contained smooth muscle cells. The segment of vessel can be recognized from the diameter, form of the cross section, and the impressions of the endothelial nuclei on the casts (Leiser and Kholer, 1984 and Leiser *et al.*, 1989). In the present study, the nuclear impressions of the endothelial cell nuclei on the wall of the vessel cast were numerous, slender to oval in shape and longitudinally oriented on the arteries and arterioles. They were relatively few, round to oval and randomly distributed on the veins and venules. In the capillaries, these impressions were clearly visible, few in number, round in shape and relatively shallow, though in other studies they have been reported as being absent (Leiser *et al.*, 1989).

The vascular architecture of the horse microcotyledon is achieved by means of a long straight artery and the capillary network invests long villi that are arranged in a partly fan-like fashion (Leiser *et al.*, 1998), being drained by a single vein (Tsutsumi 1962 and Steven, 1968). However, the present study has demonstrated that each fetal microcotyledon is vascularised through a single, centrally located artery and drained by two veins situated peripherally.

The present study is in accordance with the general principles reported by Steven and Samuel (1975) in the horse, in that the maternal circulation to the microcaruncles is in the form of long, straight branches, the microcaruncular arteries, which break over the rim and give rise to a dense vascular network on the walls of the maternal crypts, then drain to a single microcaruncular vein. Similar findings have also

been reported in the diffuse epitheliochorial placenta of the one-humped camel (Abd-Elnaeim, 1998) and pig (Leiser and Dantzer, 1988), the latter having, a very simple undulating structure. The vascular supply of the maternal side of the placenta is similar in all three aforementioned species, with the horse placenta representing the most complex situation. This is because of the ramifications of the microcaruncle into several primary, secondary and tertiary crypts, and the arterial supply for each microcaruncle occurs through relatively large microcaruncular artery that originates from the complex of uterine arteries at the level of the endometrium and myometrium as seen in the donkey uterus (Abd-Elnaeim *et al.,* 2001). In the horse, the venous outflow takes place through several venous capillary limbs that join to form several venules which converge into a large microcaruncular vein at the base of the microcaruncule. In this respect the pig placenta is similar (Leiser and Dantzer, 1988 and Dantzer and Leiser, 1994), but in the camel, the origin of the microcaruncular vein is different (Abd-Elnaeim *et al.,* 1998).

Most transplacental exchange takes place at the capillary bed (Benirschke and Kaufmann, 1995 and Leiser *et al.,* 1997a). In order to favour this area, the supplying vessels have to run as straight and directly as possible from the chorionic plate to the capillaries of the terminal villi (Benirschke and Kaufmann, 1995). The present study supports this notion, with the stem vessels and their ramifications, arterioles and venules, almost completely following the whole length of the microcotyledons, with a central and straight course for the arterial vessels and a slightly winding peripheral one for the venous vessels. On the maternal side, also, the microcaruncular arteries follow a straight feto-maternal course to the top of microcaruncles before ramifying into arterioles and then capillaries. This way of vessel ramification provides the shortest distance from the supplying vessels to the capillaries of the terminal villi on the fetal side and from the arterioles to the capillaries of the terminal crypts on the maternal side as in the bovine (Leiser *et al.,* 1997a and Pfarrer *et al.,* 2001).

The area of exchange is represented here by the thin walled vessels or capillaries of the terminal villi. For each terminal villus, a single arterial capillary limb is extended to the top constituting the capillary complex and about three venous capillary limbs converge from this complex to constitute the venules at the level of the intermediate villi. The capillaries of the terminal villi are located at the periphery of the microcotyledon and are close to the maternal microcaruncular tissue.

The capillary convolutions of these terminal villi are still simple at this stage of gestation but the author expects an increase in their complexity during the second half of gestation by increasing coiling and anastomosis. This is in agreement with the observation of Leiser *et al.* (1997b) in bovine and Abd-Elnaeim *et al.* (1998) in the one-humped camel, where they noticed an increase in the complexity of capillary convolutions with the progress of pregnancy till term.

In the present study, dilated or sinusoidal capillaries are seen particularly at the top of the fetal terminal villi and at the basal part of the terminal crypts. The presence of dilated or sinusoidal capillaries has been reported in the human (Kaufmann et al 1985 and Leiser *et al.*, 1991), bovine (Leiser *et al.*, 1997 a, b) and in the one-humped camel placenta (Abd-Elnaeim, 1998 and Abd-Elnaeim *et al.*, 1999). Sinusoidal dilatation offers an expanded endothelial surface for absorption and may slow blood flow locally (Leiser *et al.*, 1997b). This should not influence the rapidly equilibrating diffusional transplacental exchange with O_2 and CO_2 (Faber and Thornburg 1983), but may ameliorate conditions for the slow active transport of solutes (Alberts *et al.*, 1983).

There have been several attempts to classify the variety of placentae according to the geometrical arrangement of fetal and maternal capillaries or type of blood flow in the exchange area (Bartels and Moll 1964; Faber 1969; Moll 1972, 1981; Faber and Thornburg 1983; Dantzer *et al.*, 1988; Leiser *et al.*, 1989; Leiser an Koob, 1992 and Abd-Elnaeim, 1998). Several models of exchange have been defined and physiologists have calculated the efficiency of these models for diffusional transfer (Martin, 1981 and Faber and Thornburg, 1983). These models are: concurrent, countercurrent, crosscurrent and multivillous. In the horse placenta, the feto-maternal blood flow interrelationship has been suggested to be multivillous (Leiser and Kaufmann, 1994). However, in the present study, evidence from histological examination of semithin sections and microvascular corrosion casts indicates that the fetal and maternal blood flow is complicated and occurs mostly in opposite directions (Fig. 7a, 7b) suggesting that the feto-maternal blood flow interrelationship is more countercurrent "composed" than multivillous. This evident is particularly seen at the top of the terminal villi and the corresponding maternal terminal crypts. Such a countercurrent system is highly efficient, theoretically allowing arteriovenous equilibration, and is seen in the placentae of rodents, and lagomorphs as well as in equids (Kaufmann and Burton, 1994).

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LEGENDS

Fig. 1a: Light micrograph of two microplacentomes showing the fetal microcotyledons penetrating the maternal microcaruncules. The microcotyledonary artery (**MA**) appears in cross section and small microcotyledonary vein (**MV**) appears in oblique section. The microcaruncular artery (**ma**) takes a materno-fetal direction, and the microcarncular venules (**mvs**) and vein (**mv**) appear only at the base of microplacentome. Uterine glands (**UG**) are few in number, viewed here in cross sections and lead between the microplacentomes to the areola (*). X 140.

Fig. 1b: Light micrograph showing the branching pattern of a stem villus (**sv**) into intermediate (**iv**) and terminal villi (**tv**). The stem villus contains a centrally located stem artery (**SA**) and three stem veins (**SV**) which are clearly visible in the axial periphery. The intermediate villi show also an arteriole (**Ae**) in the centre and more than one venule (**Ve**) on the periphery. Note the investment of fetal placental tissues by maternal ones, and the variable distances of the interhaemal membrane (↔). X 280.

Fig. 2: Vascular cast of a fetal microcotyledon in an overview showing the supplying microcotyledonary artery (**MA**) that originated from the allantochorionic artery (**Aa**) and two relatively small microcotyledonary veins (**MV**) that formed from more than two stem veins (**SV**) to join the large allantochorionic vein (**Av**) . Note the tightly meshed capillary network of terminal villi. X 480.

Fig. 3a: Light micrograph showing part of a stem villus (**sv**), intermediate villi (**iv**) and terminal villi (**tv**) with the supplying arterial vessels. The stem artery (**SA**), and arterioles of intermediate villi (**Ae**) which divided into arterial capillary limbs (arrowhead) are shown. Note, the stem vein in cross section (**SV**). X 280.

Fig. 3b: Vascular cast of a fetal microcotyledon corresponding to figure 3a showing in overview the microcotyledonary artery (**MA**), microcotyledonary vein (**MV**) and some extravasation of the injected plastic (❄). The stem artery (SA) is in the centre of stem villous, with arterioles of the intermediate villi (↓) and arterial capillary limbs (arrowhead) of the terminal villi. Some stem veins (↑) are also visible. X 720.

Fig. 3c: Light micrograph showing a cross section through an intermediate villus at the level of division into four terminal villi (**tv**). Large size centrally located arterioles appear in cross and oblique sections (**Ae**), however, the venules (**Ve**) are present peripherally. Note, the trophoblast cells (**T**), fetal capillaries (**fc**), uterine epithelium (**U**) and maternal capillaries (**mc**). X 475.

Fig. 3d: Fetal vascular cast showing several venous capillary limbs (↗) and venules (↑) originating from the periphery of the terminal (**tv**) and intermediate (**iv**) villi. The cast also shows great capillary convolution and some dilated capillaries (❄). X 1700.

Fig. 4a: Fetal vascular cast showing tightly meshed capillaries constituting the vascular architecture of the intermediate (**iv**) and terminal (**tv**) villi. Each terminal villous capillary network is served by 3-5 capillary loops (❄). X 1100.

Fig. 4b: Frozen and partly cut fetal vascular cast viewed from the maternal side showing extension of each terminal villus by 3-5 capillary loops (✱). In each loop there is a centrally located arterial capillary limb (*). X 1500.

Fig. 4c: Light micrograph of a terminal villus corresponding to figure 4a showing a single arterial capillary limb (**ac**) extending to the top of the terminal villus and four venous capillary limbs (**vc**) appearing in the axial periphery. The fetal capillaries (**fc**) partly indent the trophoblast cells (**T**). The uterine epithelium (**U**) is thin and connected to the trophoblast cells through interdigitating microvilli (arrowhead). X 475.

Fig. 4d: Frozen-cut combined feto-maternal vascular cast showing dense fetal capillaries (**fc**) which are completely surrounded by relatively loose, maternal ones (**mc**). Note, the arterial capillary limbs (**ac**) and the venous capillary limbs (**vc**) on the vasculature of the fetal terminal villi. X 1100.

Fig. 4e: Capillary cast of a fetal microcotyledon showing variable-sized capillaries with rough surfaces and roudish nuclear impressions of endothelial cell nuclei (✱). X 4500.

Fig. 5a: Maternal vascular cast showing in an overview the vascular architecture of the endometrium that consists of different size microcaruncles. Primary (★) and secondary (✱) crypts are clearly visible. Arterioles (↑) can be also seen reaching the top of microcaruncles. X 225.

Fig. 5b: Frozen-cut maternal vascular cast showing a basket-like microcaruncle. Note, the vascular architecture of the secondary (*) tertiary (★) and terminal (⁕) crypts that correspond to the fetal stem, intermediate and terminal villi. Microcaruncular venules (**mv**) and vein (**Mv**) are also visible. X 285.

Fig. 5c: Maternal vascular cast showing microcaruncular artery (**MA**) arising from endometrial arteries (**EA**) and divided into arterioles (**Ae**) near to the top of the microcaruncle. The arterioles are subsequently divided into capillaries to constitute the vascular skeleton of the orifice or primary crypt (*), the side and base of microcarucle. There is a large microcaruncular vein (**Mv**) originating from the base of the microcaruncle. Note the honeycomb-like arrangement of capillaries on the side and base of the microcaruncle, and the impressions of endothelial cell nuclei on the arteries and vein respectively. X 700.

Fig. 6a: Maternal vascular cast showing arterioles (**Ae**) at the surface of the endometrium which ramify into capillaries of variable diameters to constitute the vascular frame of the primary crypt (★) of the microcaruncle. Three of these arterioles are seen surrounding an opening of a uterine gland (*). Note the loose arrangement of capillaries at the gland opening. X 1400.

Fig. 6b: Maternal vascular cast showing capillaries with convolutions and irregular arrangement constituting the orifice or primary crypt of a single microcaruncle. The primary crypt is divided into secondary crypts (*). X 1330.

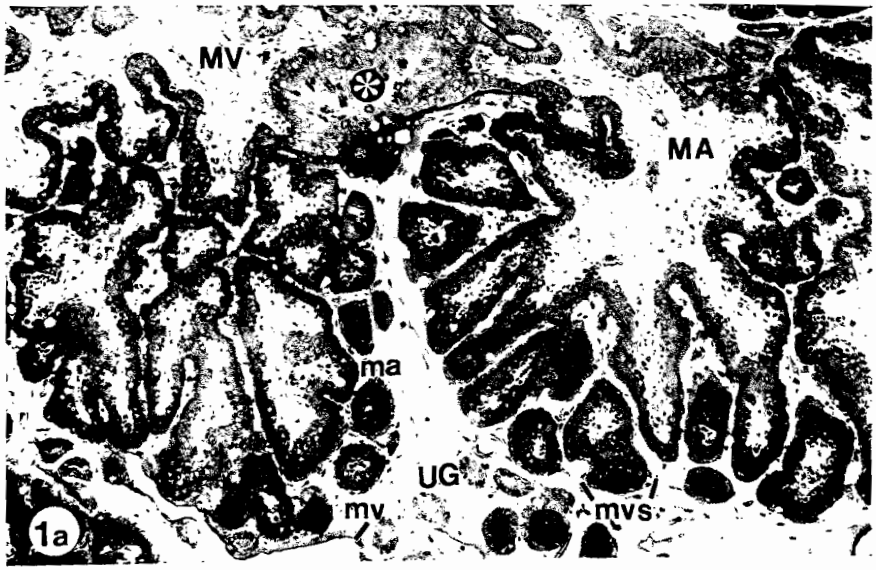
Fig. 6c: Light micrograph at the periphery of a microplacentome showing the venous capillary limb (**vc**) and venules (**ve**) originating at the base of the microcaruncle. X 280.

Fig. 6d: Frozen-cut combined feto-maternal vascular cast showing several venous capillary limbs (**vc**) originating from the base of the microcaruncle and then converging onto venules (**ve**) of variable sizes. X 900.

Fig. 6e: Maternal vascular cast of a microcaruncle in a ventro-lateral view showing capillaries (**c**), venous capillary limbs (**vc**), venules (**ve**) that converge onto a large microcaruncular vein. (**Mv**). Note, the impression of the endothelial cell nuclei on the venules and vein. X 260.

Fig. 7a: Schematic drawing of the mare microplacentome with the supplying arterial and venous vessels on both fetal and maternal side. Fetal microcotyledonary artery (**FA**), fetal microcotyledonary vein (**FV**), maternal microcaruncular artery (**MA**), maternal microcaruncular vein (**MV**), and capillary complex (**C. complex**). The arrows show the direction of blood flow in the fetal and maternal vessels.

Fig. 7b: Simple schematic drawing of the feto-maternal blood flow interrelationship in the capillary bed of the terminal villi. Fetal (**F**), maternal (**M**) and the arrows show the flow in a half of terminal villus which occurs in the opposite direction.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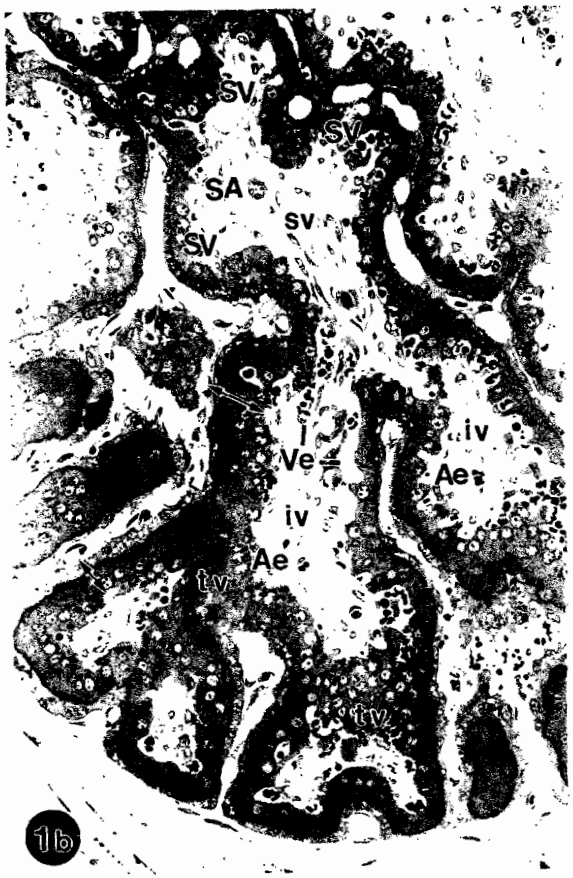
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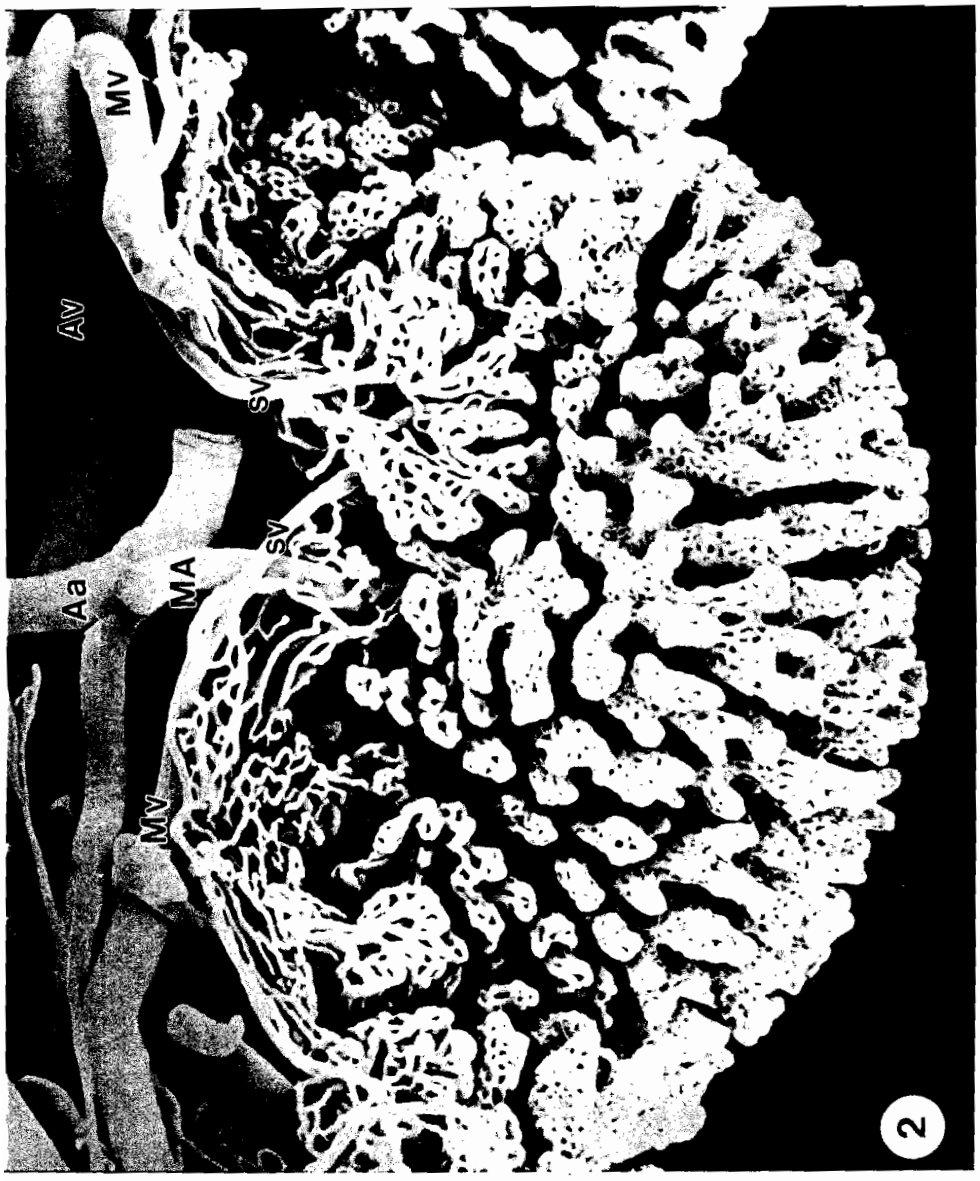
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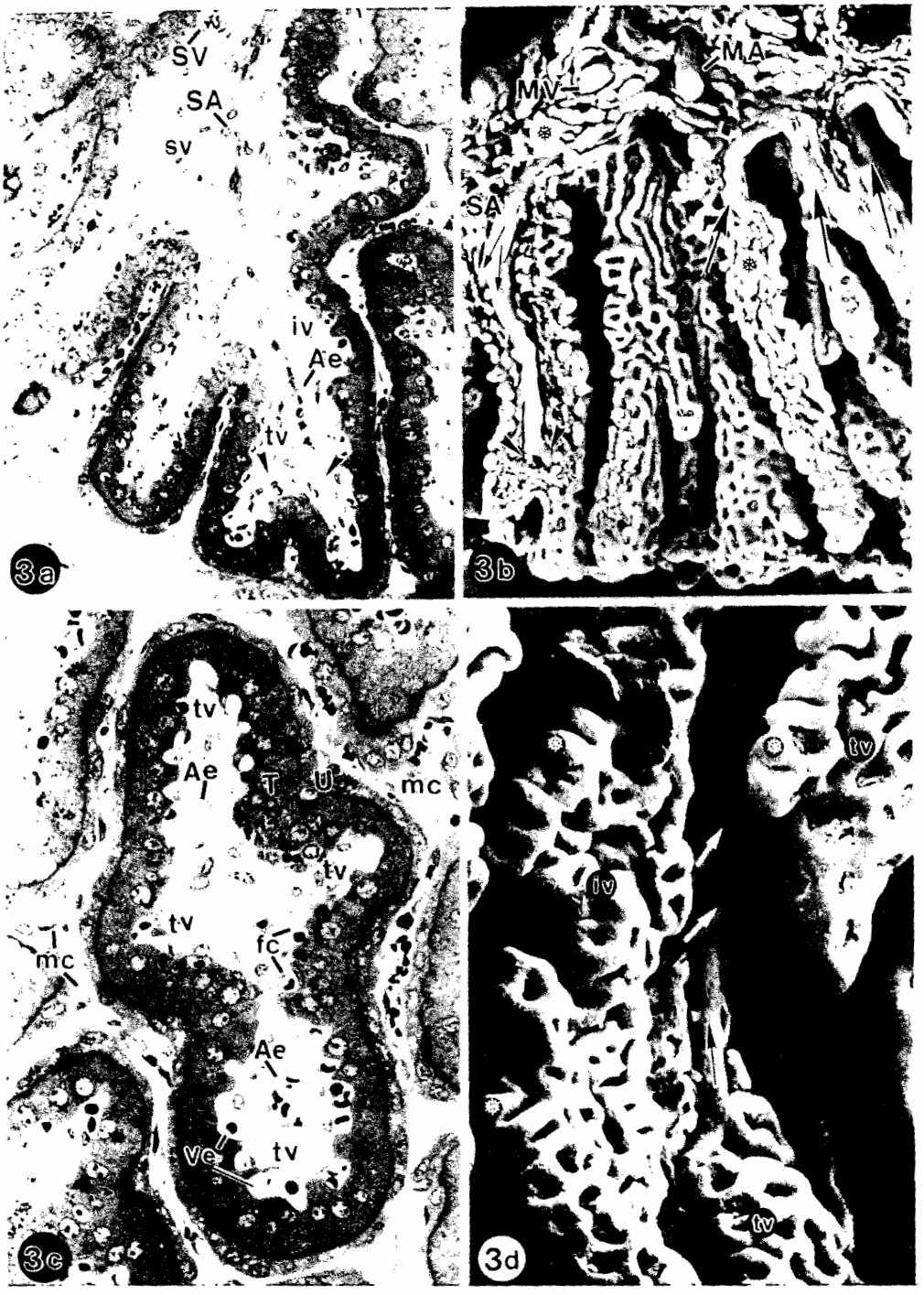
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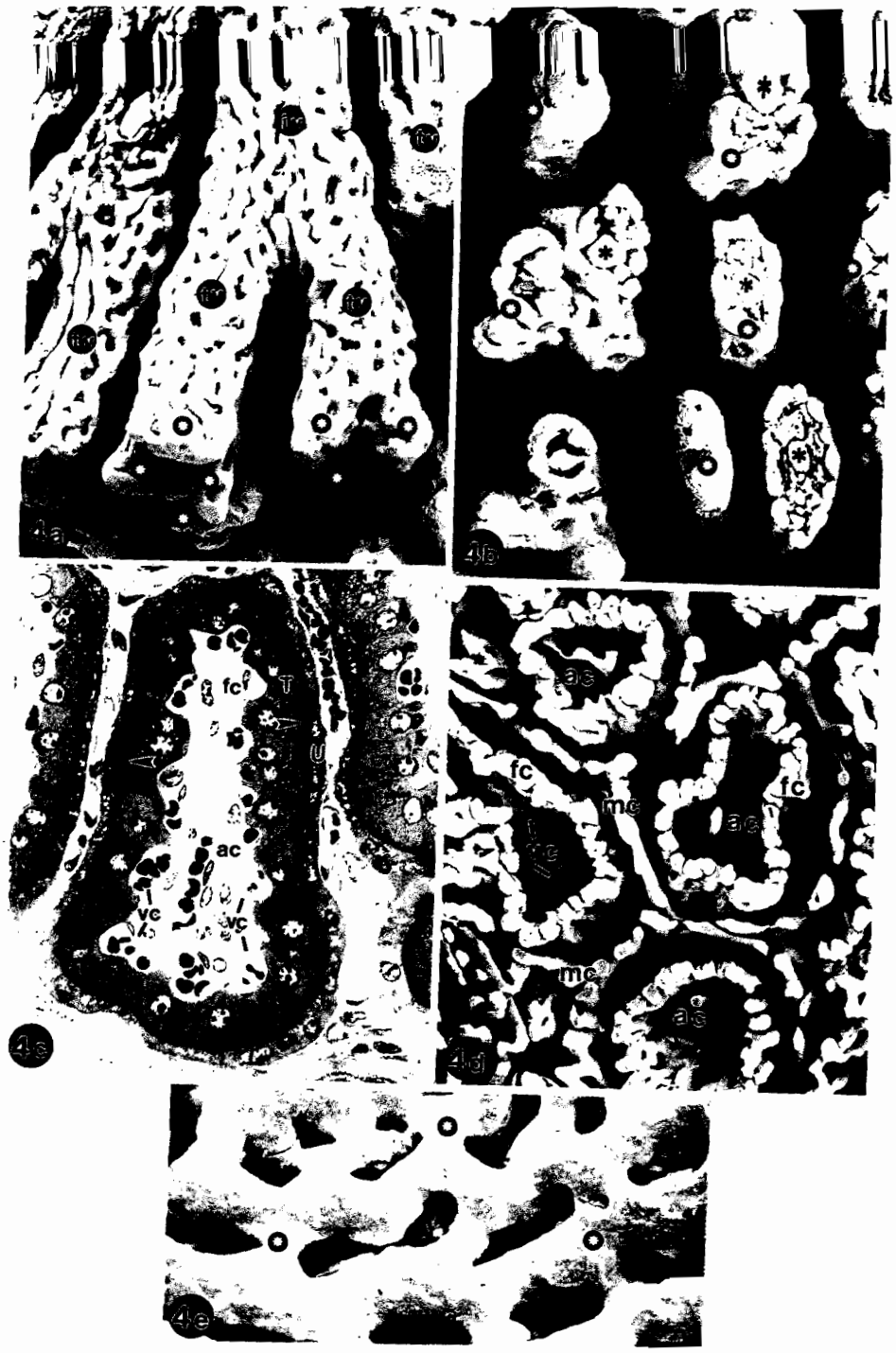
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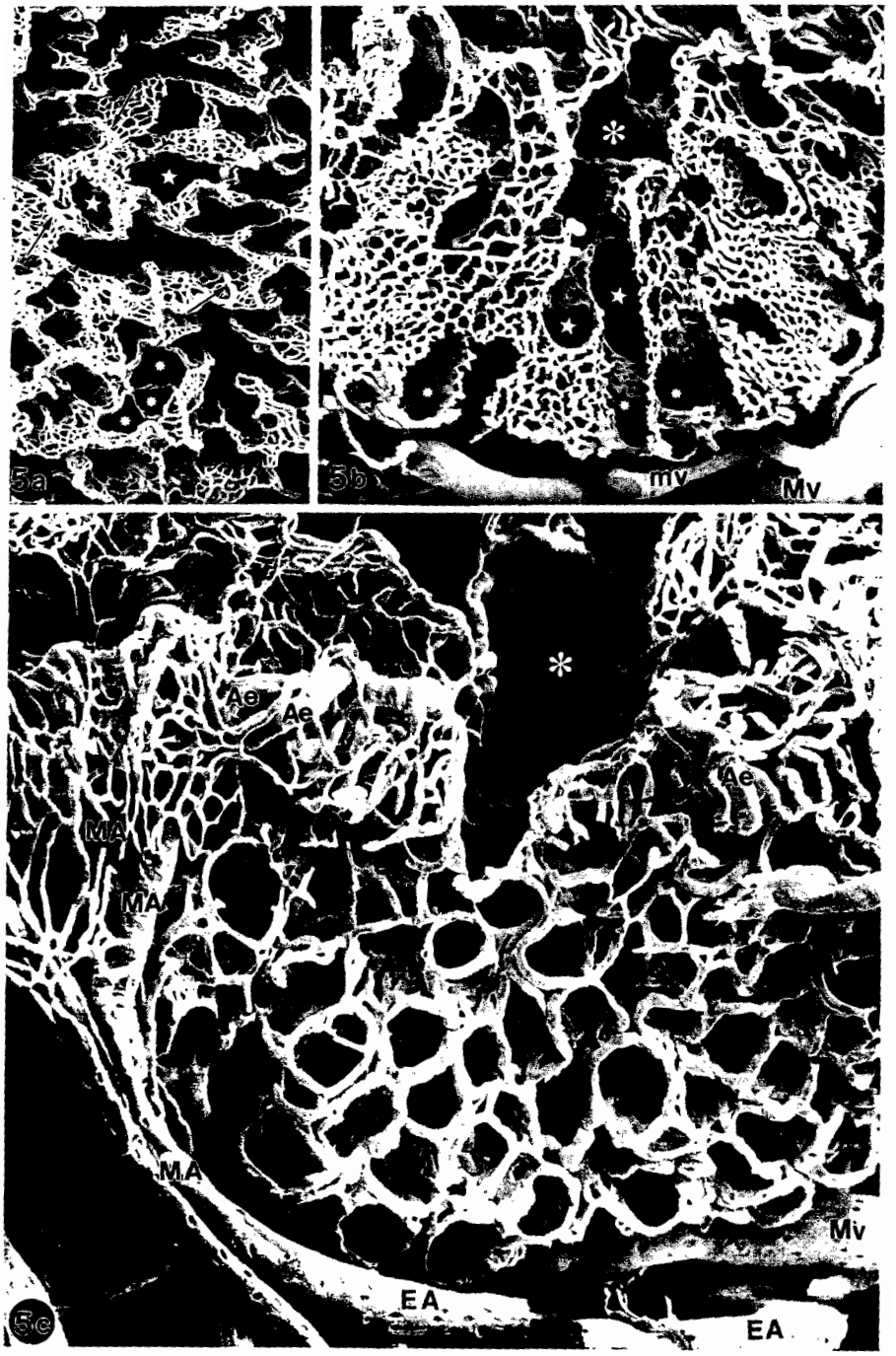
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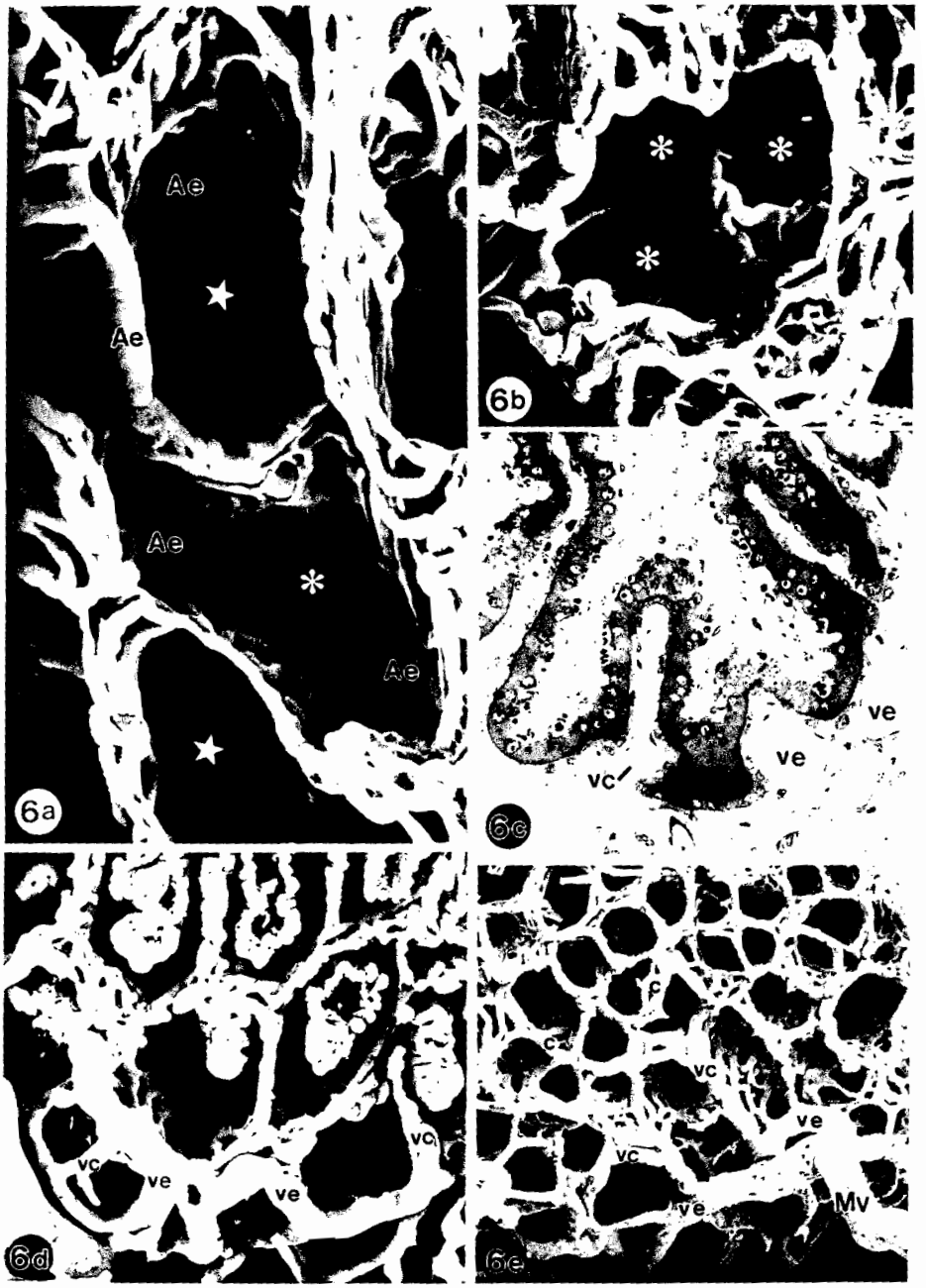
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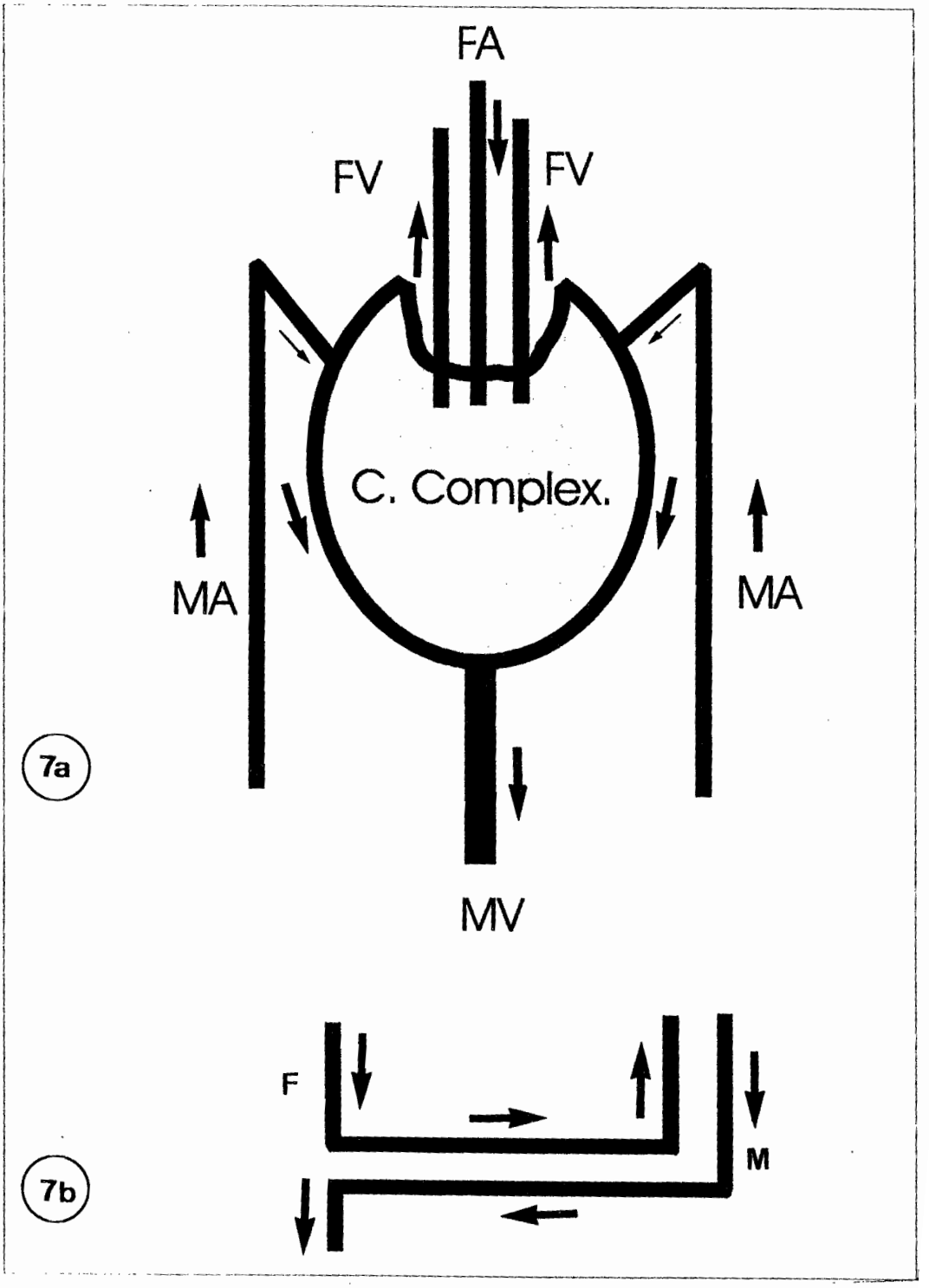
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