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Scanning Electron Microscopy of the Microcotyledonary Placenta of the Horse (*Equus caballus*) in the Latter Half of Gestation

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Scanning electron microscopy was used to examine the microstructure of the maternal and fetal placenta from 25 pregnant ponies (*Equus caballus*) throughout the second half of gestation. Samples of placenta, 2–6 cm^2 in area, were collected from the antimesometrial region of the pregnant horn at 105 days (n=1), 165–219 days (n=5), 260 days (n=3), 270–277 days (n=3), 313–337 days (n=11) and immediately after spontaneous delivery at term (n=2). The maternal microcaruncle appears to be created from a clustering of about 16 uterine crypts encapsulated in a connective tissue sheath. There is a gestational increase in the depth of the microcaruncle during the second half of pregnancy. The fetal microcotyledon appears to be formed by a clustering of individual fetal villi. The length and branching of the villi increased considerably during the last 2–3 months of gestation. Tufts of from three to six branches were seen arising from close to the base of a villous stem. Branching of the villous stem occurred not only at the base but also along the secondary and tertiary branches. There was evidence of continued branching at the tips of the villi in the few days before birth.

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INTRODUCTION

The anatomy of the placenta has been studied in a number of different ungulate species to varying degrees of detail from the gross macroscopic to the ultrastructural level (Amoroso, 1952; Ramsey, 1982; Mossman, 1987). The domesticated species of ungulate (pig, sheep, cow and horse) have received most attention (Amoroso, 1952; Ginther, 1992; Dantzer and Leiser, 1993; Wooding and Flint, 1994; Leiser et al., 1998). However, compared with the other domestic species, relatively little is known about the structure of the equine placenta, particularly in late gestation (term approx 320–360 days).

The development of the microscopic structure of the fetal equine placenta has been relatively well documented during the first 210 days (60 per cent) of gestation using light, transmission and scanning electron microscopy (Turner, 1876; Bonnet, 1889; Schauder, 1912, 1931; Drieux and Thiéry, 1949; Kurnosov, 1973; Samuel, Allen and Steven, 1974; Ginther, 1992; Bracher, Mathias and Allen, 1996; Jones et al., 1999). Several of the earlier studies concluded that the placenta was fully formed by 150–250 days of gestation (Driuex and Thiery, 1949; Kurnovsov, 1973; Samuel, Allen and Steven, 1974, 1975). Much less is known about the structure of the maternal equine placenta or about developmental changes that may occur in the fetal placenta after 250 days of gestation. The few isolated observations that have been made of equine placental structure late in gestation suggest that structural changes may continue after 250 days of gestation (Schauder, 1912; Björkman, 1965, 1970; Samuel, Allen and Steven, 1976). Certainly, the effective thickness of the trophoblast is known to be reduced during the last third of gestation (Samuel, Allen and Steven, 1976). More recently, ultrasound measurements of the gravid equine uterus have also indicated that placental growth may continue to occur between 270 days and term (Renaudin et al., 1997). Hence, in the present study, scanning electron microscopy (SEM) was used to examine the microstructure of the fetal and maternal placenta from pregnant ponies throughout the second half of gestation. Preliminary observations of some of these findings have already been published (Macdonald and Fowden, 1997; Macdonald, Chavatte and Fowden, 1999).

MATERIALS AND METHODS

Placental tissue was obtained during the second half of gestation or at term from 25 normal pony mares (*Equus caballus*).

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Samples of placenta, 2–6 cm^2 in area, were collected from the antimesometrial region of the pregnant horn at 105 days ($n=1$), 165–219 ($n=5$), 260 ($n=3$), 270–277 days ($n=3$) and 313–337 days ($n=11$) either under general anaesthesia ($n=19$; 1.5 per cent halothane in O_2 or Na pentobarbitone 20 mg/kg) or after euthanasia for humane reasons ($n=4$). In three of the late gestation mares (322, 336 and 337 days), the placental tissue was collected within 2 days of expected delivery based on mammary development (Ousey, Dudan and Rossdale, 1984). Fetal placenta was also obtained from two animals immediately after spontaneous delivery at term.

Tissues were fixed, first, either in a 10 per cent solution of buffered formalin or Bouin's fluid immediately after collection. In preparation for study in the scanning electron microscope (Philips 505), the tissues were partially or completely separated into fetal and maternal components. Secondary fixation was carried out by placing the samples in a solution of 3 per cent glutaraldehyde in 0.1 M sodium cacodylate buffer (pH=7.3) solution for 2.5 h. The samples were then immersed in 2 per cent guanidine hydrochloride and 2 per cent tannic acid overnight before being washed in 0.1 M sodium cacodylate buffer and post-fixed in 1 per cent osmium tetroxide (Murakami et al., 1977). Finally, the tissues were dehydrated through a graded series of acetones, dried in a critical point drier using carbon dioxide (Cohen, 1979), and sputter coated with 20 nm gold/palladium (Echlin, 1975).

The small size of the tissue sample collected, together with the methods used to prepare the tissues for scanning electron microscopy precluded systematic morphometry of the placental structures. However, where ever possible, maternal and fetal placental structures were counted to allow a semiquantitative analysis of placental development.

RESULTS

Maternal placenta

The uterine endometrium at 105 days of pregnancy was indented with many individual crypts which were usually arranged into irregular rows (Figure 1). The density of crypts was about 100/mm^2. The entrances to the crypts were irregularly shaped and had an opening diameter of approximately 0.05–0.15 mm. A number of the crypts appeared to be single depressions, whereas others were branched below the lumenal surface. Other crypts appeared to be clustered, and seemed to derive from a single opening. Distributed between these uterine crypts were narrower openings which represented the mouths of the uterine glands.

By about 165 days the endometrial crypts had larger openings of approx 0.08–0.25 mm diameter, and these were present in fewer numbers (<60/mm^2) on the uterine lumen (Figure 2). Although the boundaries between adjacent crypts were thinner (Figure 2), the crypt openings were organized into segregated clusters bordered by relatively broad, slightly raised areas of endometrium (Figure 3). These clusters of crypts represented small microcaruncles, which were present at a density of about 1–2/mm^2. The diameter of the irregularly round or oval base of the microcaruncle at this stage of pregnancy was about 0.5–1.5 mm. Approximately 13 openings could be seen at the base of each microcaruncle, although the numbers ranged between five and 24 ($n=14$). At 180 days approximately 16 openings into each microcaruncles could be seen, and the numbers ranged between

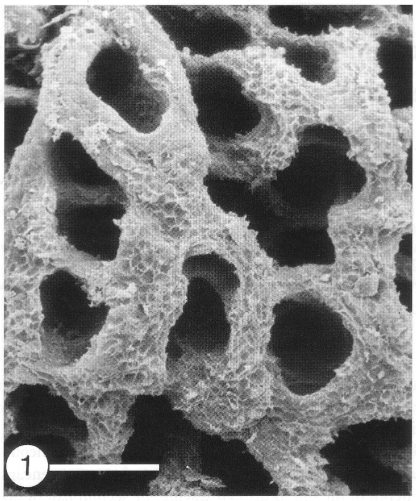

Figure 1. Scanning electron micrograph of the maternal placenta of a mare at 105 days of pregnancy. The crypts are either single or branched below the uterine lumen. Bar=0.5 mm.

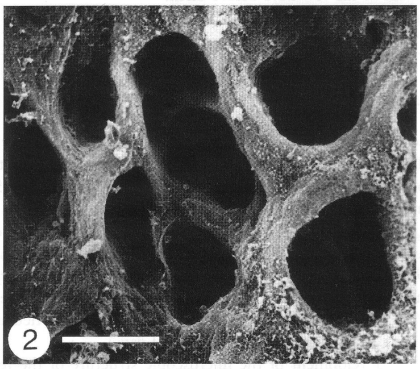

Figure 2. Scanning electron micrograph of the maternal placenta of a mare at 167 days of pregnancy. Many of the crypts appeared to be branched below the uterine lumen. Bar=0.5 mm.

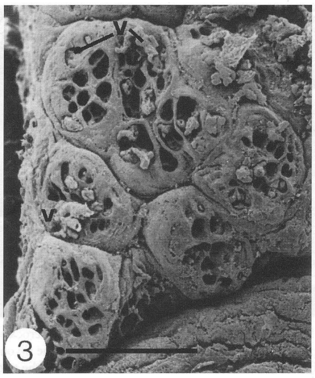

Figure 3. Scanning electron micrograph of the maternal placenta of a mare at 165 days of pregnancy. The bases of the microcaruncles are separated from one another. Note that fetal villi (v) have been trapped in several of the maternal crypts. Bar=1 mm.

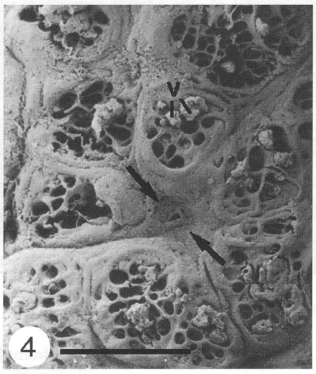

Figure 4. Scanning electron micrograph of the maternal placenta of a mare at 275 days of pregnancy. The bases of the microcaruncles are separated from one another, and surround the exit from a maternal uterine gland (→). Note that fetal villi (v) have been trapped in several of the maternal crypts. Bar=1 mm.

four and 28 (n=7). During the period of pregnancy between 165–219 days, almost all crypts were branched below the lumenal surface and individual crypts were seldom seen. Microcaruncles extended approximately 0.8 mm below the uterine lumen. Distributed between the microcaruncles were narrow openings which represented the mouths of the uterine glands.

The appearance of the endometrial surface during the period between 260 and 277 days is illustrated in Figure 4. Microcaruncles were present at a density of about 1/mm^2 and had slightly raised, irregularly shaped boundaries. The numbers of openings into each microcaruncle were difficult to

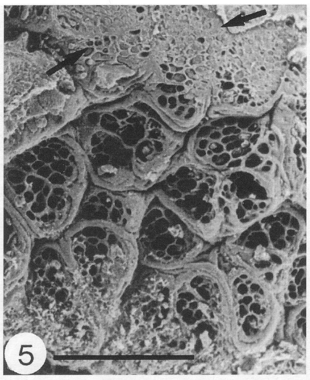

Figure 5. Scanning electron micrograph of the maternal placenta of a mare at 325 days of pregnancy. The bases of the microcaruncles are separated from one another. Note where fetal and maternal placental tissue have both been sectioned (←). Bar=1 mm.

count accurately because subdivision of the crypt occurred soon after it descended from the lumen of the uterus into the microcaruncle. Microcaruncles extended about 0.8 mm below the uterine lumen. Approximately 15 (n=5) and 17 (n=12) crypt openings into the bases of the microcaruncles were seen at 270 and 275 days respectively; the numbers for individual microcaruncles ranged from six to 23, and from eight to 38 respectively. As observed earlier in gestation, there were narrow openings from the uterine glands distributed between the microcaruncles (Figure 4).

During the period 313–337 days the microcaruncles appeared to be packed tightly together at a density of about 1–2/mm^2 (Figure 5). Endometrial boundaries between adjacent microcaruncles appeared narrower. Approximately 20 (n=7) and 35 (n=5) crypt openings into the bases of microcaruncles were seen at 325 and 329 days; the numbers for individual microcaruncles ranged from 11 to 38 at 325 days and from 17 to 51 at 329 days. Accurate counting of the numbers of openings into each microcaruncle was difficult because subdivision of the crypts occurred almost immediately after they had descended from the lumen of the uterus. Microcaruncles extended about 1.0 mm below the uterine lumen.

The three-dimensional shape of the microcaruncle is illustrated in Figure 6. The crypts exhibited a branching tubular pattern which radiated from the uterine lumen to the connective tissue boundary surrounding the ball-shaped microcaruncle. The maternal epithelial tissue was thin in appearance (see arrows in Figure 6) compared to the relatively wide diameter of the tortuous and irregularly branched crypts. Connective tissue separation of the microcaruncles was apparent when they were viewed from the myometrial surface (Figure 7). The sizes of the microcaruncles ranged from about 0.5–2.0 mm in diameter.

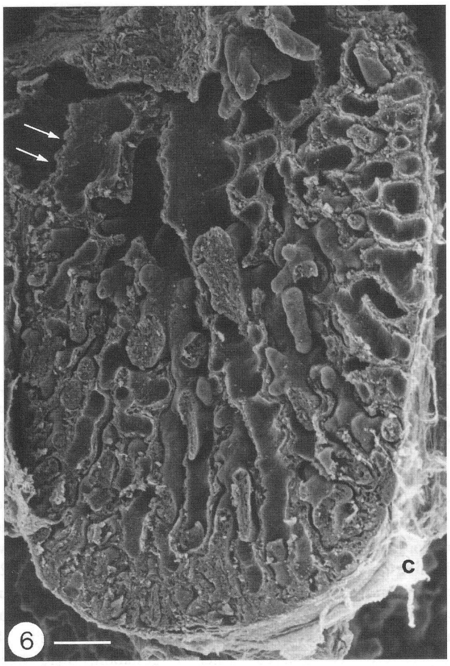

Figure 6. Scanning electron micrograph of a sectioned microcaruncle of the maternal placenta of a mare at 277 days of pregnancy. Note the connective tissue (c) support around the microcaruncle, and the thin-walled, branched tubular structure (←) representing the maternal placenta. Bar=0.1 mm.

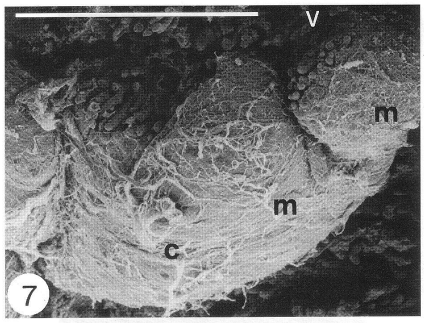

Figure 7. Scanning electron micrograph of maternal microcaruncles (m) at 329 days of pregnancy, viewed from the surface towards the myometrium after removal of the myometrium. Note the variability in size of the microcaruncles, and the subendometrial connective tissue sheaths (c) which support them. Bar=0.5 mm.

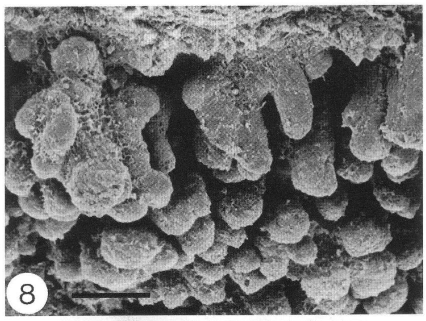

Figure 8. Scanning electron micrograph of fetal placental villi at 105 days of gestation. Bar=0.1 mm.

Fetal placenta

At 105 days of gestation the fetal placenta largely comprises rows of short (0.05–0.25 mm), bulbous villi (Figure 8). These stem from a 0.05–0.15 mm base and may be unbranched or have protrusions on their sides and apex which suggest that branching may have been taking place.

By 165–180 days the fetal placenta consists of separate clusters of villi. These range in length from <0.1 mm to approx 0.35 mm (Figure 9). The villi are sometimes leaf-like in appearance, but most often have the appearance of relatively short, bulbous rods. Swellings or other protrusions are visible along their sides and near their tops (Figure 9). Clusters of villi, single and branched, are gathered together to form microcotyledons. Within one microcotyledon as many as six villous branches were seen to arise directly from the base of one villus which was 0.15 mm in diameter.

By 260–277 days the fetal villi had increased in height and demonstrated more branching (Figure 10). The villi were arranged in dense microcotyledonary clumps (Figure 11). Those villi which were near the centre of the clump extended to about 1.0 mm in height; those at the edge of the microcotyledon were about 0.25 mm high. Tufts of from three to six branches were often seen arising from close to the base of a villous stem. Branching of the villous stem occurred not only at the base but also along the secondary and tertiary branches (Figures 10 and 11). Bulbous swellings were also present on tertiary and quaternary villous branches. Some branches subtend short stubby lateral branches, others long straight villi which divided at or near their tips.

By 313–337 days, the fetal microcotyledon was characterized by tall (1.0–1.3 mm), densely packed villi (Figures 7 and 12). Although many of these subtended branches along their lengths, others had relatively few branches. In a number of the placentae at later stages of gestation some villous branches appeared to have originated <0.2 mm below the terminal surface of the microcotyledon. Two days before delivery it was more noticeable that the tips of a number of villi were divided into two or more stubby branches (Figure 12).

The same feature was also seen in the placentae after delivery at term; the tips of a large number of villi were divided

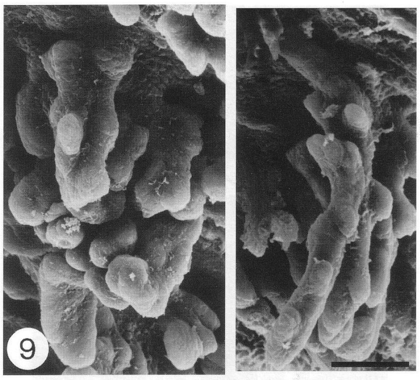

Figure 9. Scanning electron micrograph of differently sized fetal placental villi at 167 days of gestation. Bar=0.1 mm.

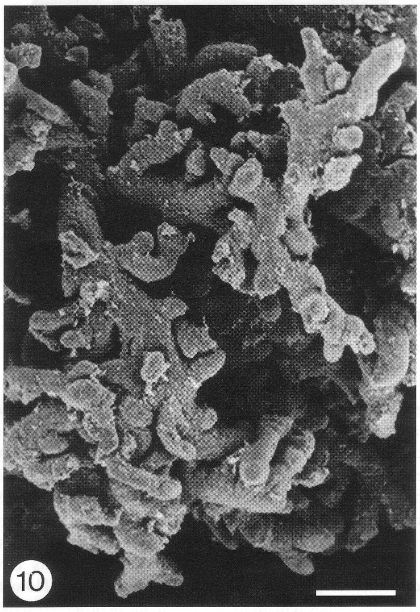

Figure 10. Scanning electron micrograph of a fetal placental villus at 260 days of gestation demonstrating primary, secondary and tertiary branching. Bar=0.1 mm.

into two or more short branches (Figures 13 and 14). The placentae at term also best demonstrated the range of sizes of the microcotyledons (Figure 15). Some appeared to have a diameter of about 3 mm, whereas others were as small as 0.3 mm. Most microcotyledons appeared to be distinct, but some seemed to coalesce into one another.

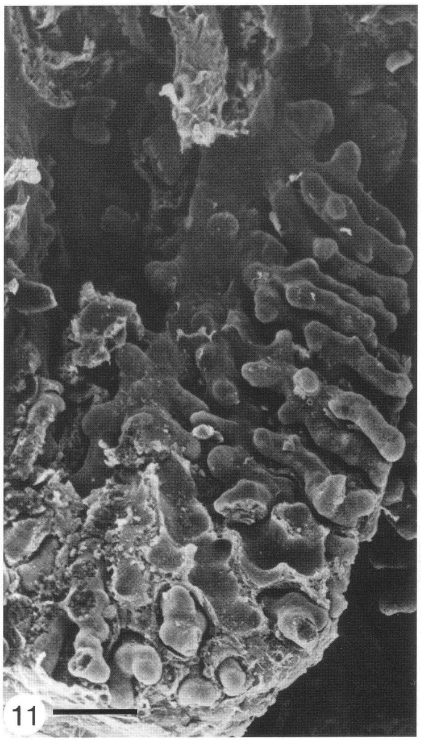

Figure 11. Scanning electron micrograph of a branched fetal placental villus within a sectioned maternal microcaruncle at 277 days of gestation. Note the number of lateral branches subtended by the main villus stem, and the short tertiary branches on these. Bar=0.1 mm.

DISCUSSION

The structure of the maternal placenta

Relatively little information has been published on the structure of the maternal placenta of the mare. The first illustrated description of the placenta of the horse was that of Fabricius (1604) in which he indicated that 'the uterine component . . . is remarkable for the exceedingly minute cavities over all its surface.' (Adelmann, 1942). The present study is the first to illustrate the appearance of these openings throughout the second half of pregnancy. It also adds considerable detail to the illustrations of microcaruncles published previously at 150 days of pregnancy (Tsutsumi, 1962) and late in gestation (Turner, 1876; Steven and Samuel, 1975; Ginther, 1992).

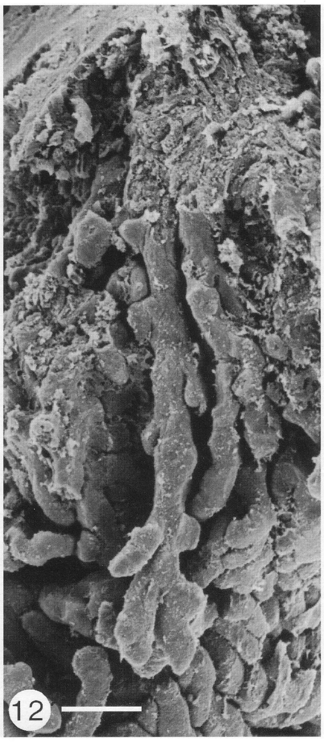

Figure 12. Scanning electron micrograph of a branched fetal placental villus at 337 days gestation and about 2 days before birth. Note the relatively few side branches on the main villus stem, and also the divisions which have occurred close to the tip of the villus. Bar=0.1 mm.

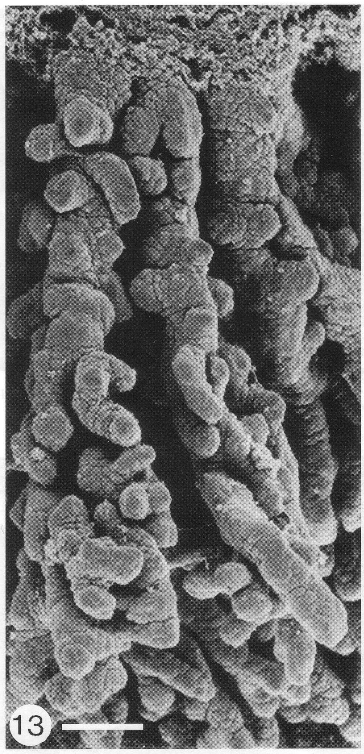

Figure 13. Scanning electron micrograph of branched fetal placental villi following birth after 342 days gestation. Note the variable thickness of the villous stems and tips due to cellular protrusions. Bar=0.1 mm.

The current finding that there is a gestational increase in the depth of the microcaruncle during the second half of pregnancy expands previous histological observations which indicated an increased depth of endometrial branching over the same period of gestation (Schauder, 1912, 1931). The thin walled, branched pattern of the uterine crypts at 277 days of pregnancy (see Figure 6) seemed to have increased during the last month of pregnancy. However, the numbers of primary crypts comprising a microcaruncle did not appear to differ much from an average of 16 per microcaruncle during the second half of gestation. The larger numbers of crypts observed towards the end of pregnancy were either the consequence of early branching of primary crypts, or due to the coalescing of adjacent microcaruncles.

The process of formation of the microcaruncles remains to be investigated in detail. In the present study there was no clear evidence of their presence at 105 days, whereas 2 months later their structure appeared to have been formed. This suggested period of development supports earlier ultrastructural studies which indicated that their formation took place between days 100 and 150 of pregnancy (Samuel, Allen and Steven, 1974). The approximately similar sizes and the relative densities of the crypts at both ages together suggest that the microcaruncle is created from a clustering of about 16 uterine crypts encapsulated in a connective tissue sheath. The size of the microcaruncle appears to be variable and its three-dimensional volume seems to increase towards term. However, systematic studies remain to be carried out in order to establish this suggestion.

The morphology of involution of the maternal placenta after delivery has been studied in some detail. The appearance of the maternal crypts during the first 12 h after separation of the fetal and maternal components of the placenta was visible as a spongy labyrinth (Steven et al., 1979). The width of the microcaruncle decreased from 0.63 mm on day 1 post partum to 0.34 mm 3 days later, and its depth decreased from 0.40 mm

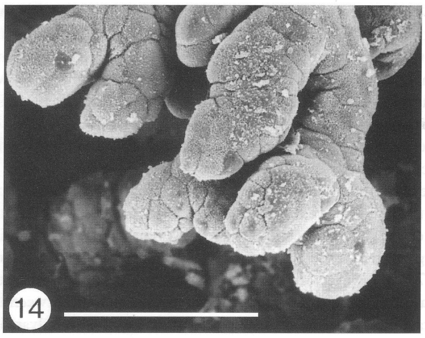

Figure 14. Scanning electron micrograph of the tips of several fetal placental villi following birth after 342 days gestation. Note the branched appearance of the tips. Bar=0.1 mm.

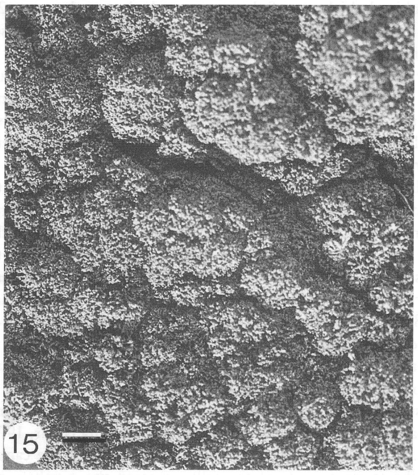

Figure 15. Scanning electron micrograph of the fetal placent following birth after 342 days of gestation. Note the variation in size of the microcotyledons, and their bushy, open appearance. The latter is probably a consequence of their release from the constraints of the maternal microcaruncle. Bar=1 mm.

to 0.15 mm over the same period (Bailey, 1983). Scanning electron micrographs of the uterine epithelium at day 1 post partum demonstrated the already convoluted appearance of the surface, with the openings of the crypts of the microcaruncles appearing as large indentations (Bailey, 1983).

Previous studies have shown that between the microcaruncles lie arcades of highly vascularized and relatively avillous spaces lined with columnar cells into which the uterine glands empty their secretions (Turner, 1876; Kurnosov 1973; Samuel, Allen and Steven, 1974; Dantzer and Leiser, 1992; Leiser et al., 1998). The uterine duct openings were observed in the present study (see Figure 4) although the fetal areolae could not be seen through the microcaruncles. A systematic evaluation of the structure and distribution of the uterine glands and the fetal areolae during the second half of pregnancy was not central to the aims of the present study and has been discussed by Schauder (1931), Samuel, Allen and Steven (1977), Dantzer and Leiser (1992) and Bracher, Mathias and Allen, (1996).

Uterine arteries run from the subendometrial vessels to the surface of the microcaruncles (Tsutsumi, 1962; Steven, 1968, 1982). Branches form around the rim of the microcaruncle before draining through the capillary networks underlying the uterine mucosa to a single vein at the base of each microcaruncle. Between days 60 and 150 of pregnancy, there is an increase in the net-like vascularization of the placenta (Samuel, Allen and Steven, 1974, 1976). However, details of the changes in structure which occur in the placental capillary net during the second half of pregnancy remain to be investigated.

The structure of the fetal placenta

There is rather more published material illustrating the form of the fetal placental villi but even these data relate primarily to the first half of gestation. The present study, therefore, largely concentrated on the developments in fetal placental structure which take place during the second half of gestation. Turner (1876) reported that the villi which almost completely covered the placental surface, were seen as brush-like clusters or tufts, like minute cotyledons. This description closely matches the earlier comments of Fabricius (1604), in which he indicated that the microcotyledonary structure of the placenta of the horse appeared as '. . . innumerable very small tubercles almost in contact with one another, scattered throughout the chorion. . . .' (Adelmann, 1942). He continues in an explanation of the coloured plate XIX '. . . the chorion is everywhere crowded with almost innumerable swarms of small tubercles corresponding to the little cavities (of the uterus), and these tubercles are nothing else than the carunculae of the chorion, which fill the little cavities of the uterus and immediately become rounded when they are separated from the uterus. These take the place of the cotyledons . . .' (Adelmann, 1942). Figure 15 in the present study illustrates this 'rounded' nature of the microcotyledon at term after it is released from the microcaruncle. It also illustrates the range in size of the microcotyledons at term which had been mentioned earlier by Schauder (1912) and Ludwig (1962). The more compact microcotyledonary form of the combined fetal and maternal placental tissues at 277 and 329 days of gestation is illustrated in Figures 6, 7 and 11.

The allantochorionic villus arises from simple, shallow, corrugated folds, which growth transforms into discrete villi (Bonnet, 1907; Schauder, 1912; Kurnosov 1973; Samuel, Allen and Steven, 1974; Ginther 1992). The simple shape of the villus at 56 days of gestation was illustrated by Ewart (1897), and Samuel, Allen and Steven (1974) reported that by day 100 there was secondary folding of the primary villi. Kurnosov (1973) reported numerous arborescent villi by day 95–96, and Ewart (1897) illustrated a branched villus at 112 days. The villi at 105 days of gestation in the present study were variously shaped, some having a simple villous structure and a number either branched or with protrusions on their sides (see Figure 8). Some of the latter may be seen in scanning electron micrographs of fetal placenta from a thoroughbred mare at 80 days published by Bracher, Mathias and Allen (1996).

Microcotyledons could be seen at 80 days of gestation in the Thoroughbred (Bracher, Mathias and Allen, 1996). However, individual microcotyledons could not be differentiated from one another in the non-gravid horn because of the density of villi. Previously Schauder (1912, 1929) had reported that microcotyledons could be observed from about 98 days of gestation. No evidence of them was found at 105 days in the present study, nor were they reported at 100 days by the ultrastructural studies of Samuel, Allen and Steven (1974). However, as with the studies of Bracher, Mathias and Allen (1996), the specific site of placental sampling, even in the gravid horn, may have influenced the incidence of these structures in early gestation.

Previous studies indicated that an approximately two-fold increase in the length of the fetal villus occurred between about days 105 and 170 of gestation (Ewart, 1897; Samuel, Allen and Steven, 1974), and this was confirmed in the present study. Branching of the villus at 167 days, though short, was also more apparent than at 105 days (see Figures 8 and 9). It was also clear in the present study that by day 165 of gestation, clusters of villi had been organized into microcotyledons. Other recent studies have reported that microcotyledons are well developed by day 150 of gestation (Samuel, Allen and Steven, 1974). Although, to date, there have been no detailed studies of the development of the microcotyledons, there are two contrasting views of their formation. They may develop either by branching of a single villus stem (Schauder, 1912; Amoroso, 1952; Ludwig, 1962; Leiser et al., 1998) or by clustering of individual villi (Bujard, 1907; Samuel, Allen and Steven, 1974). The results of the current studies of maternal tissues support the latter view but further investigations during the period of gestation between days 50 and 150 are required to resolve this issue.

By day 260 of gestation the length and branching of the villus had increased considerably over that seen 100 days earlier. An earlier SEM study demonstrated elongation of the villi and secondary branching at 200 days of gestation (Bracher, Mathias and Allen, 1976). By day 260 this process of elongation and branching had progressed further, with tertiary and quaternary branches of the villi and signs of the potential for additional branching. Although the villous branching at about this stage of gestation may be seen in the illustration published by Turner (1876), and inferred from the transmission electron micrograph of a pony placenta at 250 days (Samuel, Allen and Steven, 1975), its three-dimensional structure is seen for the first time in the present study.

In the present study, there was also evidence of maintained growth in the branched appearance of the fetal villi during the last 2–3 months of gestation. The villi appeared to be somewhat longer after 317 days than at 260 days, and there was evidence of continued branching at the tips of the villi, particularly in the last few days before birth (see Figure 14). Previous studies have not reported these structural developments near the end of gestation. This may be due, in part, to the wide individual variation in gestational length in the mare but may also reflect the belief that development of the microcotyledons is complete by the seventh month of gestation (e.g. Drieux and Thiéry, 1949; Björkman, 1965; Kurnosov, 1973). However, ultrastructural studies have demonstrated that there is a progressive decrease in the distance between the fetal and maternal placental blood streams with advancing gestational age (Steven and Samuel, 1975; Samuel, Allen and Steven, 1976) but, even in these studies, no observations were made after 300 days of gestation. Ultrasonographic measurements of the gravid uterus have shown a 5 mm increase in the combined thickness of the uterus and placenta between 270 days and term (Renaudin et al., 1997). The current observations indicate that placental thickness increases by about 20 per cent over this period of gestation. Since this would account for only 1 mm of the measured increase in the combined uterine and placental thickness, hypertrophy of the myometrial and connective tissue is also likely to occur in the equine uterus during the last 2–3 months of pregnancy.

In the present study the measurements made of the dimensions and density of the fetal villi and maternal crypts were only semi-quantitative. Previous studies have shown that the size of fetal villi within a single placenta varies with the position in the uterus with longer villi in the non-pregnant horn (Schauder, 1912; Whitwell and Jeffcott, 1975; Mossman, 1987). Although the placental samples used in the present study were taken consistently from the antimesometrial region of the pregnant horn, the possibility of slight regional variations in villi size cannot be excluded entirely. Fixation of tissues also produces a variable degree of shrinkage which may be as much as 35 per cent in the equine placenta (Baur, 1977). In addition, during late gestation, the dense, tree-like canopy of the terminal ends of the villi obscured the numbers and surface area of the villous branches beneath. For these reasons, it was difficult to make an accurate quantitative assessment of the morphological changes that occur in the equine placenta during late gestation (Bracher, Mathias and Allen, 1996). Nevertheless, the dimensions of the fetal villi and microcotyledons observed in utero close to term in the present study were similar to those reported previously for material collected immediately after delivery (Schauder, 1912; Ludwig, 1962).

The stimulus to fetal branching

The stimulus to further branching of the fetal villus, particularly close to term, is not known but may be related to the endocrine changes that occur in the fetus and mare during late gestation. Hormones such as oestrogen, progestagens, relaxin and prostagladins are all known to affect cell growth and proliferation in reproductive tissues during pregnancy. They also affect the density and organization of the collagen and elastin fibres in tissues such as the cervix and uterus (Rajabi, Solomon and Poole, 1991; Sato et al., 1991; Bienkiewicz, Welfel and Kus, 1996; Bani, 1997; Luque et al., 1998). In the pregnant mare, oestrogen and prostaglandin levels are low during late gestation and rise rapidly in the last 24–48 h of gestation (Silver, 1994). In contrast, maternal progestagen concentrations rise during the last 20–30 days of gestation and then fall in the final 2–3 days before delivery (Rossdale et al., 1991). Similarly, relaxin, which is derived from the placenta in the horse (Stewart et al., 1982), is elevated in concentration in maternal plasma during late gestation (Stewart and Stabenfeldt, 1981). These prepartum hormonal changes may modify the biochemical composition of the layer of connective tissue covering the microcaruncles (see Figures 6, 7 and 11) and, thereby, allow further branching of the fetal villi by removing some of the maternal constraint on growth. Alternatively, they may act directly to stimulate proliferation of the trophoblast. Certainly, the branching observed at the tips of the fetal villi at delivery and during the period of mammary development just before birth occurs at a stage of gestation when the maternal hormone concentrations are changing most rapidly (Silver, 1994).

The changes in the microscopic structure of the placenta between 260 days and term have important implications for placental function during late gestation as the microcotyledons are the main site of transplacental nutrient transfer in the mare (Silver, Steven and Comline, 1973). Between mid and late gestation, fetal body weight doubles and the fetal requirement for glucose increases two to three-fold (Platt, 1984; Fowden and Forhead, 1999). The gestational increase in the length and branching of the fetal villi and maternal crypts will therefore increase the surface area available for placental exchange and help meet the nutritional demands of the fetus during late gestation when it is growing most rapidly in absolute terms. However, the extent to which changes in placental structure can be related directly to altered nutrient provision remains to be determined.

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