Placenta (2003), **24**, 598–617
doi:10.1016/S0143-4004(03)00102-4

Placentation in the African Elephant (*Loxodonta africana*): II Morphological Changes in the Uterus and Placenta Throughout Gestation

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Paper accepted 27 March 2003

The gross and microscopic development of the zonary endotheliochorial placenta in the African elephant was studied in 22 gravid uteri that ranged in gestational stage from 0.5 to 20.6 months. The conceptus only ever occupies one horn of the uterus and is associated with 2–5 large corpora lutea that persist in the ipsilateral ovary throughout gestation. Initially, the trophoblast in the equatorial region of the conceptus completely replaces the lumenal epithelium of the endometrium to which it is apposed. Blunt upgrowths of endometrial stroma then develop, each closely invested by trophoblast, and containing the capillaries that will vascularize this maternal component of the resulting placental band. With advancing gestation the lamellate stromal upgrowths increase markedly in length and become much thinner, thereby bringing the trophoblast into intimate contact with the endothelium of the maternal capillaries. They also become folded or pleated to increase the total area of intimate feto-maternal contact. At the lateral edges of the placental band the lamellae bend over towards the endometrium to form a blind cleft. Leakage of blood into this area creates haemophagous zones in which phenotypically specialized trophoblast cells phagocytose the blood components.

The presence of large resorbing blood clots and circumferential scars in the uteri of three post parturient animals initiated the hypothesis that, when the standing elephant gives birth at term, the passage of the 120 kg fetus through the vagina may wrench the placenta off the endometrium by severing its very narrow maternal placental hilus. The resulting intrauterine haemorrhage may then play a role in preventing further conception for around 2 years.
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INTRODUCTION

Almost 40 years ago J. S. Perry and the late E. C. Amoroso gave an elegant and detailed description of the fetal membranes and placenta of the African elephant (*Loxodonta africana*), using material gathered from eight pregnant uteri harvested during routine culling in Uganda in the 1950s and 1960s (Perry, 1953; Amoroso and Perry, 1964; Perry, 1974). Prior to that time, information on pregnancy in the elephant had been limited to sporadic accounts in captive animals of births, untoward abortions and post mortem findings following death during pregnancy (Owen, 1868; Chapman, 1880; Assheton, 1906; Cooper, Connell and Wellings, 1964). Since then, attention has tended to focus more on ovarian function and peripheral plasma hormone profiles (Hanks and Short, 1972; Ogle, Braach and Buss, 1973; McNeilly et al., 1983; Brannian et al., 1988; Plotka et al., 1988; de Villiers, Skinner and Hall-Martin, 1989; Hodges et al., 1994, 1997; Heistermann, Trohorsch and Hodges, 1997; Heistermann, Fieß and Hodges, 1997; Hodges, 1998) in cycling and pregnant females. In addition, Laws (1969) published an excellent account of the ecology and population dynamics of elephants in East African wildlife parks, Moss (1983) and Poole (1996) have written extensively on elephant behaviour, reproductive parameters and family life and van Aarde, Whyte and Pimm (1999) recently described the dynamics of the elephant population in Kruger National Park.

Amoroso and Perry (1964) described the differentiation of the extraembryonic membranes in the young elephant conceptus, including the unusual division of the allantois into four distinct compartments. They noted that the initially spherical conceptus lodged within one of three or four lateral grooves of the star-shaped endometrial lumen in one uterine horn and that, as the conceptus expanded, the outermost trophoblast cells appeared to erode and replace the lumenal epithelium of the endometrium over a considerable area of the chorion. Subsequently, a typical zonary placenta began to develop in

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just the equatorial region of the now ovate conceptus and this became essentially similar in architecture to the zonary placenta of canids, felids and the mink (Amoroso, 1952; Enders, 1957; Mossman, 1987; Wooding and Flint, 1994). Furthermore, the elephant placenta also had a diffuse haemophagous region under the lateral edges of the placental band in which trophoblast cells phagocytosed leaked maternal blood components (Amoroso and Perry, 1964). This haemophagous zone was similar to that found in the hyaena placenta but differed significantly from the sharply defined haemophagous sacs that develop in the placentae of the dog and mink (Steven, 1975; Steven and Morriss, 1975; Burton, 1982).

We were afforded a similar opportunity to study the development and architecture of the elephant placenta by examining and sampling gravid uteri recovered from adult female elephants being culled professionally in the Kruger National Game Park in South Africa. We recorded the gross appearance and general development of the zonary placenta and other fetal membranes and related these to gestational stage based on fetal weight (Craig, 1984). We also recovered pieces of placenta, fetal membranes, fetal gonad, endometrium and other fetal and maternal tissues for endocrinological studies (Allen et al., 2002), and for microscopic and immunohistochemical examinations. In this paper we summarize our gross, light microscopic and ultrastructural observations and, through these, attempt to describe the development and functioning of the elephant fetoplacental unit throughout gestation.

MATERIALS AND METHODS

Recovery of specimens

For 2 weeks in each of three successive years (1993 to 1995 inclusive) three of the authors joined the annual cull of elephants in the Kruger National Game Park in the Western Transvaal region of South Africa. An accurate aerial census of total elephant numbers in the whole park was carried out annually and, based on the results, between 350 and 450 elephants were selected for culling in one of four main divisions of the Park (van Aarde, Whyte and Pimm, 1999). Each day one whole family group, which usually consisted of one matriarchal female, 4–6 of her adult daughters and their respective calves, was selected for killing. This was carried out by an experienced marksman working from a Bell Ranger helicopter that was flown in circles to herd all the family members into a tight group. The marksman then fired a compressed air dart pre-loaded with a neuromuscular paralysing agent into the rump of each elephant to immobilize it on the ground within 50–70 sec. When all the animals had been felled in this manner the helicopter hovered above them to enable the marksmen to kill each individual with a single brain shot from a 410 calibre rifle. The ground team, waiting in vehicles about 1 mile distant, was then called in by radio to exsanguinate the carcasses by severing the carotid artery and to commence the task of evisceration. The entire reproductive tract from each sub-adult and adult female was brought to a makeshift laboratory established at the edge of the culling area where it was photographed and dissected to recover the required placental tissues and conceptus fluids.

Tissue sampling

During the 3 years of the study a total of 83 reproductive tracts removed from adult (>15 years old) and sub-adult (estimated 5–14 years of age) females were examined, of which 58 were gravid (70 per cent). Each tract was laid out flat, with the ventral surface uppermost, on a table-top or on the ground, and photographed. The ovaries were examined for the presence of follicles and/or corpora lutea before they were taken for other experiments. When the uterus was judged to be non-pregnant, an attempt was made to open one horn to examine the surface of the endometrium for signs of previous pregnancies or pathological changes. Biopsies of endometrium were taken from some uteri and either snap frozen in OCT embedding medium (Raymond Lamb Laboratory Supplies, Eastbourne, Sussex, UK) in a bath of isopentane in liquid nitrogen, or fixed in Bouin's fluid, buffered 4 per cent formaldehyde or 1 per cent glutaraldehyde plus 3 per cent paraformaldehyde for light and electron microscopic processing.

Gravid uteri in the earliest stages of gestation were carefully dissected over the conceptus bulge in an attempt to expose the conceptus intact. If successful the conceptus was photographed before a sample of allantoic fluid was collected using a syringe and 21 gauge needle. Following rupture of the allantochorion, small pieces of endometrium with embryonic membranes attached (very early specimens; estimated 15–40 days' gestation), or pieces cut across the entire band-like placental zone (slightly later specimens; estimated 2–9 months gestation), or pieces cut from the middle, lateral edges and maternal placental hilus areas of the placental zone (late specimens; >9 months' gestation) were either fixed, snap frozen in OCT for immunohistochemistry, or snap frozen as a chunk of tissue in liquid nitrogen for in situ hybridization, all as described by Wooding et al. (1996). The embryo or fetus was removed and weighed and, in selected cases, its intra-abdominal gonads were recovered through a flank laparotomy incision to establish its sex. With a few selected uteri, pieces of placental zone and fetal gonad were immersed in cold phosphate buffered saline (PBS, pH 7.2) and driven quickly to the laboratory for steroid hormone conversion and gonadotrophin extraction experiments, as described by Allen et al. (2002). In all, placental tissue and/or endometrium was collected, processed and examined microscopically from 22 gravid uteri containing fetuses that weighed 0–98 kg and were estimated, on the basis of fetal weight, to be between 15 days and 20.6 months of gestation (Table 1).

Table 1. Elephant conceptuses from which samples of fetal membranes and maternal endometrium were recovered for gross, microscopic and ultrastructural studies of the development of the placenta.

Cull number	Year of cull	Fetal sex	Fetal weight	Estimated gestational age*
E 17	1993	–	–†	15–40 d
E 18	1995	–	–†	15–40 d
E 58	1994	–	1.6 g	~120 d
E 86	1995	–	2.0 g	~125 d
E 36	1994	–	4.0 g	~135 d
E 42	1993	–	5.15 g†	~140 d
E 29	1994	–	20 g	5.5 m
E 34	1995	–	93 g	6.1 m
E 50	1994	–	120 g	6.2 m
E 44	1995	M	136 g	6.3 m
E 55	1994	–	189 g	6.5 m
E 78	1994	F	220 g	6.6 m
E 54	1995	M	240 g	6.7 m
E 78	1995	F	650 g	7.55 m
E 57	1994	M	1.9 kg	8.85 m
E 42	1994	F	7.4 kg	11.3 m
E 74	1995	F	17 kg	13.5 m
E 19	1995	M	33 kg	15.7 m
E 62	1995	F	44 kg†	16.8 m
E 21	1995	M	50 kg	17.4 m
E 56	1994	F	70 kg	19.1 m
E 78	1995	F	98 kg	20.6 m

* The gestational age of foetuses weighing <10 grams was calculated using the formula, $t=105\ w\frac{1}{3}-(w\frac{1}{3}+0.193)^{-3}+140$ where t=gestation length in days and w=foetal weight in kg. The gestational age of foetuses weighing >10 grams was calculated using the formula $t=106\ w\frac{1}{3}+138$ where t=gestation length in months and w=fetal weight in kg. Both formulae derived from Craig (1984). M=male, F=female. d=days, m=months.

† Placenta examined ultrastructurally.

Histology and electron microscopy

Tissues for light microscopy were immersed in Bouin's fluid for 48–72 h before being trimmed and transferred to 70 per cent methanol for transport back to England. Alternatively, they were fixed in 4 per cent buffered formaldehyde and maintained in that fixative until processed. Tissues for electron microscopy were immersed in 1 per cent glutaraldehyde/3 per cent paraformaldehyde before being transferred after 48 h to 0.5 M phosphate buffer for transport and storage. The snap frozen samples were transferred to a liquid N_2 'dry shipper' tank for transport.

The Bouin's and formol saline fixed samples were dehydrated by passing them through increasing concentrations of alcohol followed by xylene. They were then embedded in paraffin wax and sectioned at 5–8 μm for staining with haematoxylin and eosin (H&E). The glutaraldehyde/paraformaldehyde fixed samples were processed for ultrastructural studies through osmium and ethanol into araldite (Wooding et al., 1996). Semi-thin and ultra-thin araldite sections were cut and stained with toluidine blue for light microscopy or uranyl acetate and lead citrate for electron microscopy.

RESULTS

Anatomy of the uterus and ovaries

The bicornuate uterus shows some similarity to that of the horse in that the two horns are relatively straight and they diverge laterally from each other within the broad ligament (Figures 1 and 2a). Beyond this gross appearance, however, there are some marked contrasts. First, the body of the elephant uterus is shorter than its counterpart in the horse and is therefore not unlike that of the dromedary camel (Figure 1; Skidmore, Wooding and Allen, 1996). This means that the horns, joined to each other by a short intercornuate ligament as in the ruminant uterus, run parallel in an anterior direction for a greater distance before they diverge laterally away from each other towards the ovaries (Figures 1 and 2a). Second, a great deal of fat is deposited in, or is closely associated with, the broad ligament (Figure 2a) which itself is much thicker and stronger than its equine equivalent. The broad ligament also becomes noticeably puckered and 'rope-like' in appearance where it gathers itself together and passes anterio-laterally to become incorporated in the ovarian and lateral uterine ligaments which, in turn, are anchored to the dorsolateral wall of

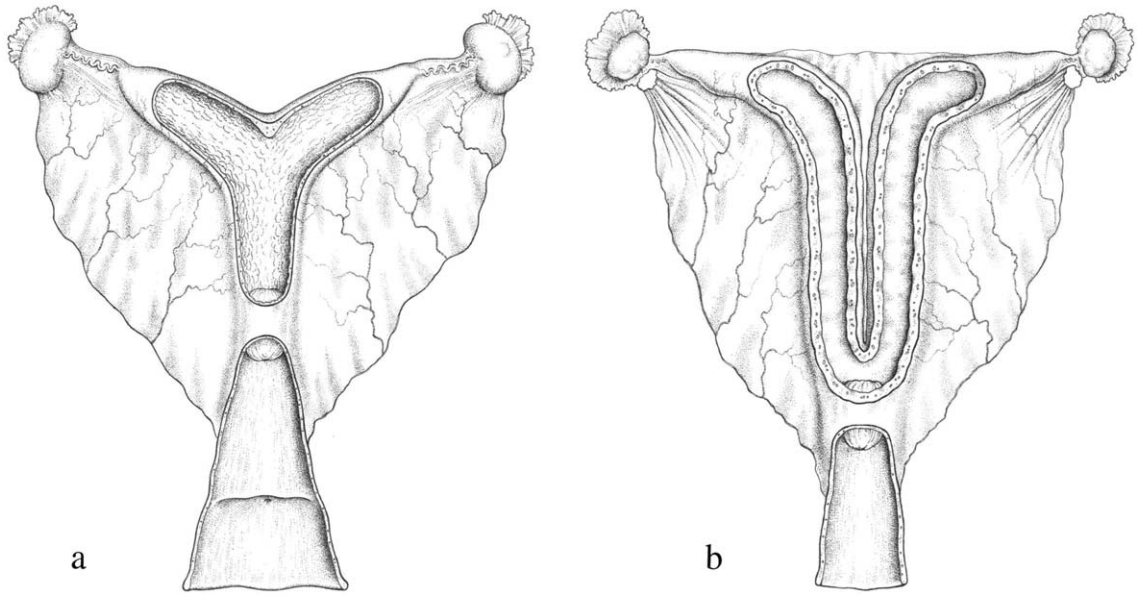

Figure 1. Stylized comparison of the reproductive tracts of the horse and elephant. In the former (a), the long uterine body and lateral facing uterine horns are lined by a soft velvety endometrium that is densely packed with exocrine secretory glands and is arranged in longitudinal folds and circular rugae. The uterus is suspended in a relatively uniform broad ligament containing the supplying blood vessels and nerves and each uterine horn is connected to a large kidney-shaped ovary by a long and convoluted oviduct that terminates in a relatively small fimbria covering only the indented ovulation fossa on the medial surface of the ovary. The mature pre-ovulatory Graafian follicle commonly reaches a diameter of 4–5 cm and the corpus luteum which forms in its place after ovulation is of a similar diameter. In the elephant (b), by contrast, the very short uterine body quickly divides into two separate horns that are embedded in a much thicker and more fibrous broad ligament, are joined by a short intercornual ligament, and run parallel to each other in an anterior direction for some distance before they curve sharply and run laterally towards each ovary. The endometrium is paler, smoother and much firmer than that of the horse, contains many fewer secretory glands and is not arranged in folds and rugae. The uterine lumen is tightly closed and, in the straight posterior portions of the horns, exhibits lateral clefts opening off a central core. The ovaries are relatively small, fibrous and nodular and they are joined to the uterine horns by a short, straight oviduct, the infundibulum of which forms a large mucosa-lined pouch or sac that completely envelops the ovary. The thickened broad ligament, which contains considerable deposits of fat, becomes puckered anteriorly as it merges into the lateral uterine and ovarian ligaments which together form a tendinous rope-like attachment to the dorsal wall of the abdomen.

the abdomen (Figures 1 and 2a). This pronounced puckering and thickening of the tissues near the point of insertion clearly adds strength to the broad ligament, enabling it to support a gravid uterus that must weigh in excess of 150 kg during the final stages of gestation (Perry, 1953; Laws, 1969; Craig, 1984).

In non-pregnant young adult elephants, the outermost serosa, underlying myometrium and innermost endometrium are collectively so fibrous, tough and tonic that it was impossible to insert a sharp knife or scalpel blade into the lumen of the uterus near the uterine body and slit one horn open along its length, as is achieved easily in other large mammals like the horse and cow. In these tough elephant uteri the knife point would slip in and out of the lumen in an uncontrolled manner as forward pressure was applied. When the lumen was actually breached the pale and thick endometrium would immediately bulge out through the incision as if under considerable pressure (Figure 2b). A transverse section across the whole uterus posterior to the lateral divergence of the two horns showed the close proximity but clear separation of the horns and it highlighted the folding of the endometrium to form the star-shaped uterine lumen (Figure 2c), as described previously by Perry (1964) and Amoroso and Perry (1964). Histological sections of the endometrium in this region showed very tight apposition of the lumenal epithelial layers within the lateral clefts of the star to give a histological picture more reminiscent of a rabbit uterus than of an animal as large as an elephant (Mossman, 1987).

The adult elephant ovary is rather small in relation to overall body size and is fibrous and nodular in general appearance, not unlike that of the pig (Figure 2d). In situ it is completely enveloped by the infundibulum of the oviduct which is incorporated in the ovarian bursa and thereby forms a distinct serosal pouch which completely envelops the ovary (see Perry, 1953 for details of the development). The internal surface of this sac-like infundibulum is lined by a red, velvety mucosa (Figure 2d). A number of the uteri recovered from both pregnant and non-pregnant animals in the older age groups exhibited papillomatous outgrowths on the outer serosal surface (Figure 2e). These measured 0.5–2.0 cm in diameter and were raised 0.5–1.0 cm above the surface. Although they could be anywhere on the exterior of the uterus, there was a definite tendency for them to be clustered near the tip of a uterine horn, and thereby in close proximity to the rather exuberant mucosa of the oviducal infundibulum (Figure 2e). Initially it was thought that these 'uterine warts' might be explants of mucosa shed from the oviducal infundibulum that had been created in a similar manner to the explants of refluxed menstrual endometrium that grow on the serosal surfaces of the uterus and neighbouring intestines in women suffering from endometriosis (Leyendecker et al., 1998).

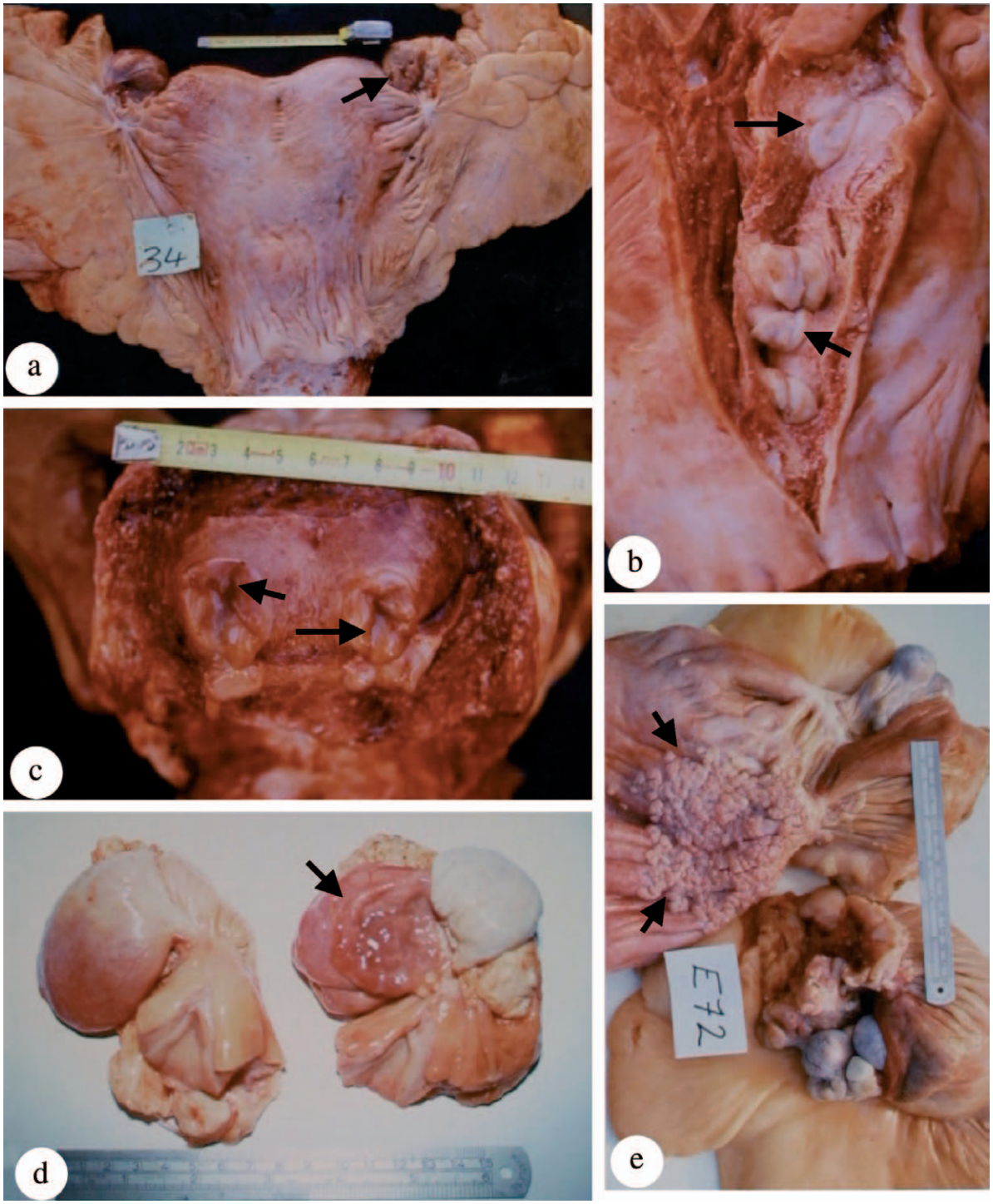

Figure 2. (a) Reproductive tract of a non-pregnant adult elephant viewed from the dorsal surface. The very short uterine body and parallel uterine horns running anteriorly are difficult to discern within the very thickened and fibrous broad ligament. Note the large fat deposits at the periphery of the broad ligament which becomes noticeably puckered as it merges with the lateral uterine and ovarian ligaments. The infundibulum of the left oviduct has been everted to show its reddened and luscious-looking mucosal lining (arrowed). The right ovary is still completely enveloped within its infundibulum. The bulge of the cervix is visible at the base of the uterus. (b) Failed attempt to slit open one horn of the uterus recovered from a non-pregnant young adult female. Where the lumen has been entered the very pale endometrium bulges out under considerable internal pressure (arrows). Note the thickened and very fibrous appearance of the uterine wall. (c) Transverse section across both uterine horns made just anterior to the uterine body in a pregnant female carrying a fetus weighing 1.6 grams. Note the extreme compression of the central uterine lumen and the formation of star-shaped lateral clefts (arrowed). Note also the great thickness and toughened, fibrous architecture of the uterine wall. (d) A pair of ovaries and the anterior tip of each uterine horn from a non-pregnant adult female. The ovary on the left is still completely enveloped within its infundibulum of the oviduct while that on the right has been extruded to reveal the oedematous and velvety mucosal lining of the infundibulum (arrowed). (e) The tips of the gravid and non-gravid uterine horns of a pregnant female carrying a 650 g fetus, showing a dense accumulation of wart-like outgrowths on the external serosal surface of the gravid horn (arrowed). Unusually, two large plum-like corpora lutea, each measuring 3–6 cm in diameter, are present in both ovaries. The combined volume of the luteal structures force the ovary to become extruded from the infundibulum.

However, subsequent histological examination showed them to be no more than simple exfoliative outgrowths of the uterine serosa, with no evidence of any mucosal components.

In all the ovaries recovered from both non-pregnant and pregnant elephants, the biggest Graafian follicle encountered was 1.5 cm in diameter (Figure 3a). Clusters of smaller (0.3–0.8 mm) follicles were observed occasionally but single, large, clearly pre-ovulatory follicles were very rare. On the other hand, the ovaries attached to every pregnant uterus, even those in the very earliest stages of gestation, always exhibited between 2 and 6 large corpora lutea (CLs) that measured 3–6 cm in diameter and hence were much bigger than the progenitor follicles (Figure 2e and Figure 3b). These large luteal structures were usually, athough not invariably (Figure 2e), bunched together on one ovary situated ipsilateral to the gravid uterine horn. When sectioned they exhibited an homogeneous pale brown-coloured luteal parenchyma with few signs of fibrous trabeculae or other supportive elements (Figure 4a). Thus, a fascinating and as yet unexplained discrepancy seems to exist between the paucity and relatively small size of Graafian follicles in the ovaries of pregnant elephants and the much larger, multiple CL that are always present in pregnant animals at all stages of gestation (see Perry, 1953; Hodges, 1998 and Allen et al., 2002 for discussions).

Gross development of the conceptus

Pregnancy was first 'guessed at' in two uteri as a tiny bulge 3 cm in diameter situated at approximately the anterio-lateral reflection of the uterine horn ipsilateral to an ovary containing the characteristic multiple large CLs of pregnancy. Despite careful dissection down through the thick and tough uterine stroma, in both cases the conceptus ruptured with a sudden release of a few millilitres of embryonic fluid, to leave a thin, barely discernible, pale choriovitelline membrane attached to the underlying endometrium. No embryonic elements could be distinguished in either of these uteri and biopsies of endometrium with attached membranes were recovered randomly for fixation and snap freezing. These two embryonic sacs were arbitrarily assigned a gestational age of 15–40 days.

Beyond this very early stage the conceptus bulge became increasingly obvious as a discrete ovate swelling positioned at the lateral reflection of the gravid uterine horn (Figure 3c). The persisting compressive tonicity of the tough uterine wall still hampered dissection down on to the conceptus and it made the chorion or allantochorion bulge outwards through the incision the moment it was exposed (Figure 3d). It was now possible to visualize and sample the pale, ribbon-like thickening in the equatorial region of the ovoid conceptus that constituted the developing placental band (Figure 3e). Furthermore, the division of the allantois into separate compartments could be discerned by peering through the slightly opaque allantochorion (Figure 3e).

With further advances in gestation, the conceptus bulge became bigger and more like a rugby ball and thence a large melon in shape, filling the whole of the gravid horn but never extending into the uterine body or the non-gravid horn (Figure 4a). Furthermore, the general tonicity of the uterus seemed to lessen, thereby simplifying dissection through the tough uterine wall so as to expose the conceptus intact (Figure 4a and d).

The placental band, seen initially as a pale, annulate thickening around the conceptus (Figure 3e, 5.15 g fetus), broadened as a result of lateral growth and became increasingly reddish-brown in colour as gestation progressed. In most instances it formed a complete, unbroken ribbon of tissue with relatively uniform brown-coloured lateral edges resulting from the formation of the haemophagous zone (Figure 4a, 220 g embryo), but some specimens showed definite regions of narrowing of the placental band, and even some clear breaks in it (Figure 4b, 240 g fetus). In one conceptus, a portion of the placental band gave the appearance of having formed in a longitudinal, rather than latitudinal, orientation on the surface of the conceptus (Figure 4b). The unattached allantochorion on either side of the placental zone had a pale, opaque, slightly roughened appearance, beneath which the outline of the separate allantoic compartments could be distinguished (Figure 4b).

During the second half of gestation, the cut surface of the placental band consisted of large areas of homogeneous reddish-brown tissue made up of the closely packed materno-fetal lamellae separated by fibrous trabeculae carrying the larger blood vessels (Figure 5a and b, 98 kg fetus). The lateral edges of the band were closely apposed to the uterine epithelium and were much darker brown in colour as a result of the leakage of maternal blood to form the haemophagous zones (Figure 4d, 1.9 kg fetus, Table 1 and Figure 5b, 98 kg fetus). A surprising feature in all the gravid uteri, which became more pronounced as gestation progressed, was the relative narrowness of the maternal placental hilus that formed the only attachment of the conceptus to the endometrium (Figure 4a and e, 189 g and 1.9 kg fetuses respectively). This structure, which measured only 2–4 cm in width during the first half of gestation, formed as a result of the original upgrowth of stromal villi from the lumenal surface of the endometrium during development of the placental band, and it carried the increasingly dense plexus of maternal blood vessels involved in vascularizing the band (Figure 4e). However, despite the relatively enormous expansion of the band throughout gestation, to become >12 in wide and some 4–6 in thick at term (Figure 5b, 98 kg fetus), the original 2–4 cm maternal placental hilus gained very little additional width. Thus, it remained a remarkably narrow channel or tract that carried a growing concentration of enlarging uterine blood vessels, much like the pampiniform plexus supplying the testis in a male. The pedicle could be easily severed using conventional scissors and doing so around the whole conceptus allowed the latter to simply roll out of the uterus (Figure 4e). Also visible during the second half of pregnancy were numerous white, wart-like structures dotted all over the surfaces of both the allantoic sacs and the thickened white amnion enveloping the fetus (Figure 4d). These too were described previously by Amoroso and Perry

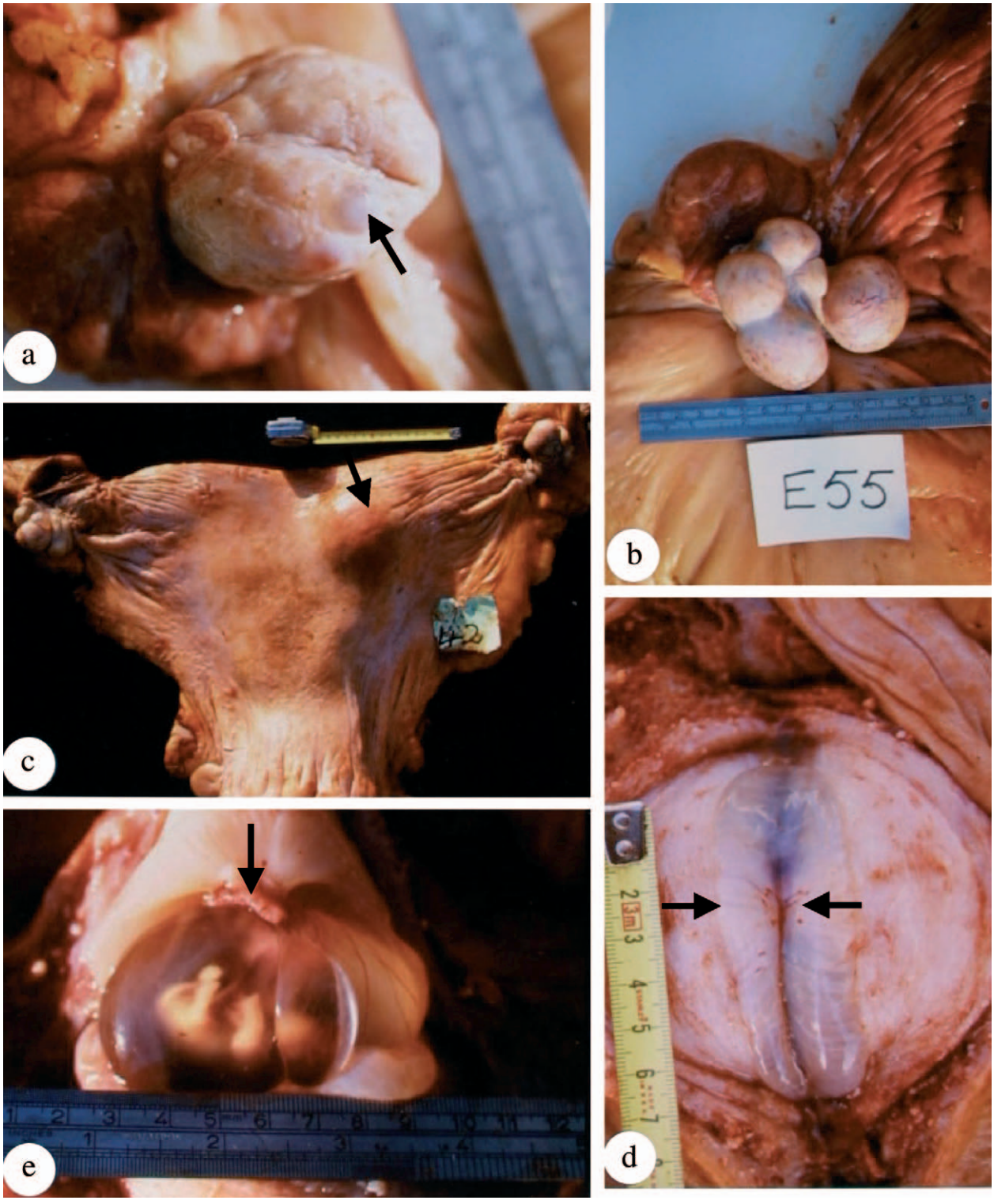

Figure 3. (a) Ovary adjacent to the non-gravid uterine horn in a pregnant female carrying a 1.9 kg fetus. Note the single Graafian follicle of approximately 1.5 cm diameter protruding from the external surface (arrowed). The ovary associated with the gravid horn had the usual multiple CLs. (b) Four large pregnancy CLs, each measuring 3–6 cm in diameter, clustered on the ovary ipsilateral to the gravid horn of a pregnant elephant carrying a fetus weighing 189 g. (c) Gravid uterus viewed from the ventral surface. The discrete conceptus bulge (arrowed), containing a fetus weighing 5.15 g, is situated at the point of lateral divergence of the left uterine horn. (d) Opened conceptus sac containing a 1.6 g fetus. Note the pressurized bulging of the pale, toughened endometrium with the attached embryonic membranes. The pale outline of the progenitor placental band is just visible (arrowed), in the middle of which primitive embryonic and placental blood vessels can be seen. (e) The opened conceptus bulge shown in Figure 4c revealing the 5.15 g fetus lying on its back. The separate allantoic locules can be seen and the transversely sectioned developing placental band is visible at the top of the conceptus (arrowed).

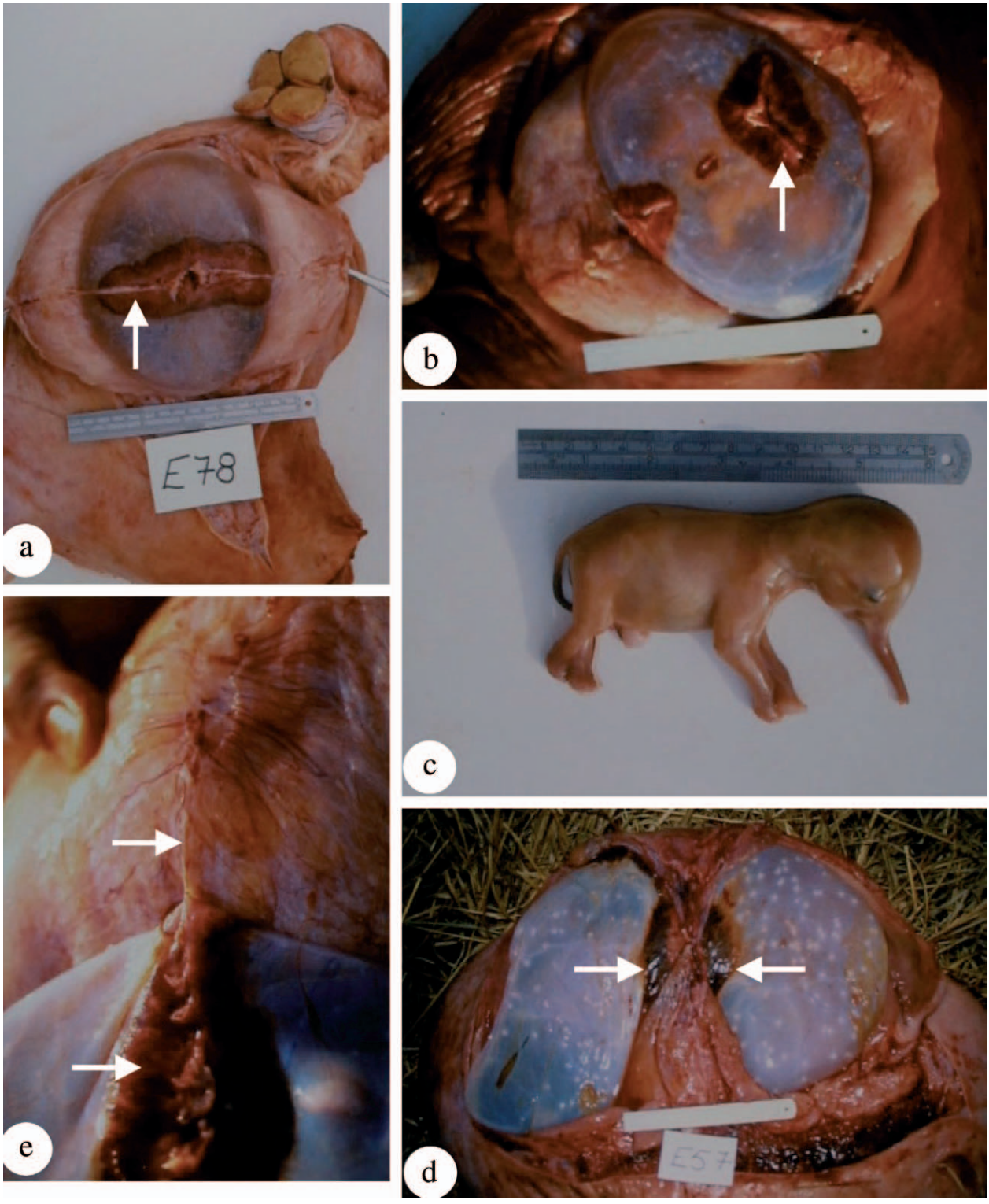

Figure 4. (a) Gravid horn of the uterus of a pregnant female carrying a 220 g fetus opened to reveal the ovate conceptus now firmly attached to the endometrium by the thin fibrous-looking maternal placental hilus running in the centre along the length of the thickened and reddish-coloured placental band (arrowed). Note the dark brown-coloured haemophagous zones at the lateral edges of the placental band and the pale brown-coloured homogenous luteal tissue in the two large sectioned corpora lutea in the ipsilateral ovary. (b) Gravid horn opened to reveal an intact conceptus carrying a fetus weighing 240 g. Note the distinct gaps in the placental band and the skewed longitudinal, rather that latitudinal, arrangement of one piece of the placental band in the equatorial region of the ovate conceptus (arrowed). (c) The 240 g male fetus recovered from the conceptus shown above in Figure 4b. (d) Partly opened uterus showing the intact conceptus in situ with a fetus weighing 1.9 kg. Note the dark brown colouration of the haemophagous zone at the lateral edges of the placental band (arrowed) and the numerous white allantoic pustules beneath the chorion. (e) Close up view of the maternal placental hilus being severed from the endometrium (arrows) to allow the conceptus containing the 1.9 kg fetus shown in Figure 4d to roll out of the uterus. Note the relative narrowness of the maternal placental hilus and the great concentration of endometrial blood vessels feeding into it.

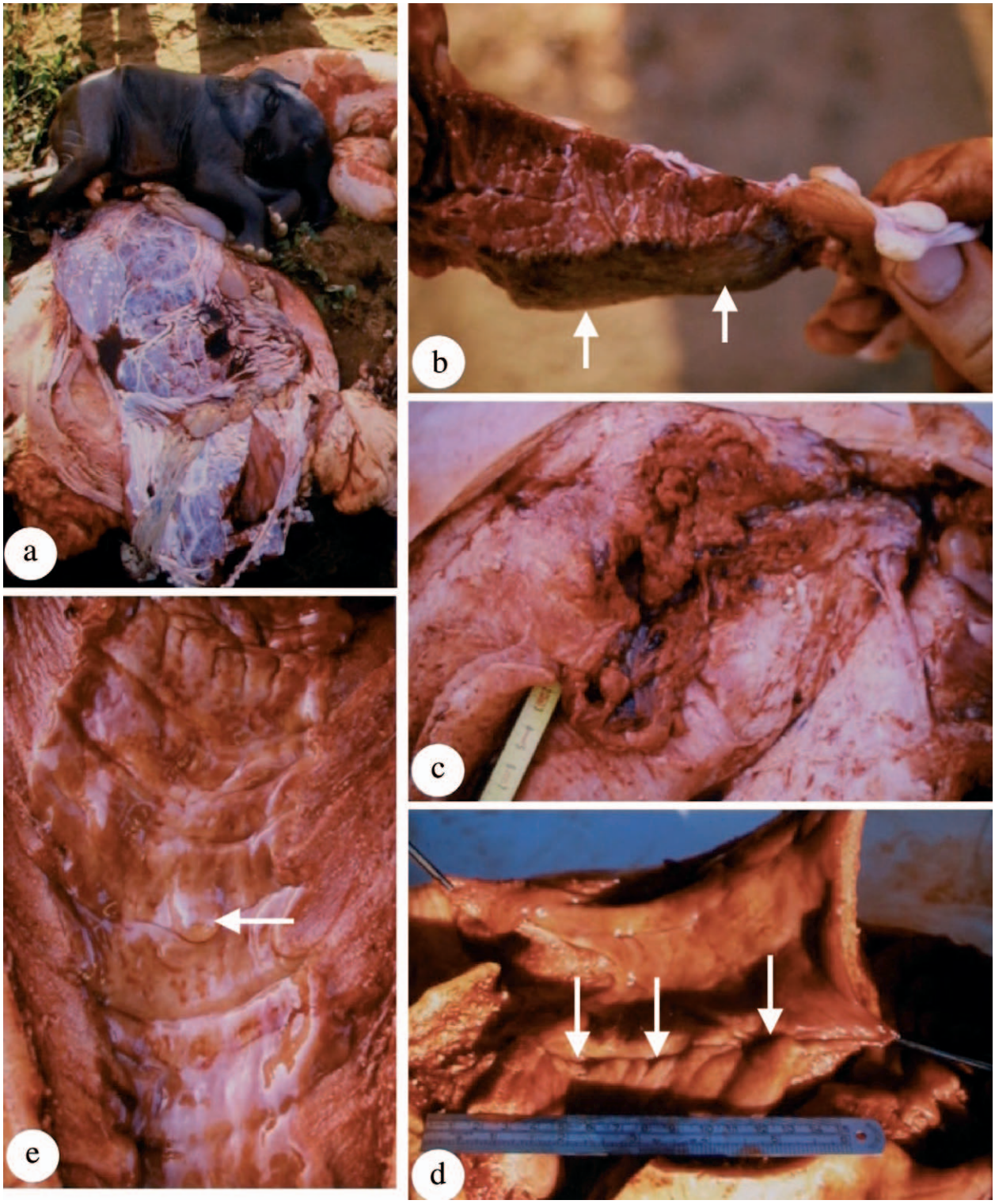

Figure 5. (a) Opened uterus showing a near-term fetus of 98 kg attached by a rather oedematous umbilical cord to the very broad and thickened placental band. Allantoic pustules are visible on the inner surface of the allantois. (b) Cross-section of one side of the placental band of the 98 kg fetus shown in Figure 5a. Note its general similarity in appearance to liver tissue and the brown colouration of the ventral surface due to leakage of maternal blood in this haemophagous zone (arrowed). (c) The previously gravid uterine horn of one of 3 post parturient uteri, opened to show a large resorbing blood clot firmly attached to the endometrial surface and occluding the uterine lumen. This elephant had a calf at foot that was estimated to be 6 months of age. (d) Previously gravid uterine horn of the third post-parturient elephant (no associated calf at foot) showing a pronounced circumferential trench-like scar (arrowed) which suggests the site of attachment of the maternal placental hilus from the previous pregnancy. Note the brown colouration of the endometrium due, presumably, to accumulation of haemosiderin in the tissues. (e) Endometrial surface of one uterine horn in a very aged (>60 years) non-pregnant female showing 6 concentric circumferential lines which perhaps indicate the sites of attachment of each maternal placental hilus from previous pregnancies. A lymph filled endometrial cyst is seen in the centre of the photograph (arrowed).

(1964) who showed them to be simple, biologically insignificant expansions of mesoderm covered by the relevant epithelium.

Three of the non-gravid uteri examined were clearly from recently parturient cows. Two had calves at foot which, on the basis of crown–rump length and height at the withers (Laws, 1969; Craig, 1984), were judged to be 6 and 8 months old, respectively; no calf could be associated with the third cow. These uteri differed markedly from all others examined in that they felt slack and generally atonic when handled externally. Accordingly, it was much simpler to expose the uterine lumen with a conventional butcher's knife and thereby reveal oedematous endometrial folds that were decidedly brownish-yellow in colour, due presumably, by analogy with the post parturient equine endometrium, to accumulated haemosiderin in the stromal and epithelial layers (Figure 5e). And in both uteri from the females with identifiable calves, the lumen of the previously gravid horn contained a large haematoma in the process of resorbing (Figure 5c). It was evident that considerable haemorrhaging into the lumen had occurred, presumably at the time of the preceding parturition, and presumably as a result of the placenta being ripped off its narrow, heavily vascularized maternal placental hilus by the sudden downward pull of the full-term fetus passing rapidly through the long sloping vagina of the standing mother (Moss, 1983; Poole, 1996).

In the third post-parturient uterus there was no sign of blood but the presence of a pronounced narrow trench-like scar around the circumference of the gravid horn appeared to indicate the site of attachment of the previous maternal placental hilus (Figure 5d). And in the uterus of one very aged (estimated >60 years old) non-pregnant female, six similar but less pronounced, circumferential scars in the rather oedematous, yellow-brown coloured endometrium were thought to indicate the attachment sites of the placental hilus in previous pregnancies (Figure 5e; Laws, 1969).

Histological development of the placenta

In the two very early specimens recovered that were arbitrarily estimated to be at 15–40 days post conception, a choriovitelline membrane, composed of an outer layer of cuboidal-to-low columnar, epithelioid trophoblast cells and a loosely attached thin inner layer of elongated endodermal cells, could be identified in three quite different and presumably stage-specific relationships to the maternal endometrium. In sections of tissue recovered from approximately the equatorial region of the vesicle, the trophoblast layer was closely apposed, but not really attached, to the lumenal epithelium of the endometrium and was just starting to extrude blunt outgrowths of trophoblast cells into the epithelial layer. In some areas these outgrowths had become elongated cellular fingers that were forcing their way beneath the epithelium and lifting it off the basement membrane in sheets (Figure 6a, no embryo visible; Table 1). And in other areas still, the lumenal epithelium over a considerable area of the endometrium had been replaced completely by trophoblast (Figure 6b, no embryo visible and Figure 7a). Isolated clumps of degenerating epithelial cells engulfed by the trophoblast layer could be seen (Figure 6a) and in some places the trophoblast cells had traversed a short distance down the lumen of an endometrial gland, lifting the glandular epithelial cells off the basement membrane as they progressed (Figure 6b, no embryo visible). Further along the endometrium in the same section a well delineated area of simple, blunt upgrowths of endometrial stroma containing capillaries could be seen indenting the investing trophoblast layer (Figure 6c, no embryo visible and Figure 7b). These were likely to have been the progenitors of the enormously elongated stromal fingers that would eventually comprise the maternal component of the established placental band. Thus, in these two early specimens, a transition from attachment to, erosion of, and finally complete replacement of, the endometrial epithelium by trophoblast could be distinguished, and these processes then led on to the very early growth of trophoblast-invested upgrowths of endometrial vasculature and stroma (Figure 6c). At neither of these early, nor at later, stages of placental initiation and growth did histological examinations indicate the presence of maternal cellular immune response to the trophoblast, such as infiltration of the endometrium by lymphocytes or other white blood cells.

A considerable jump in development existed between these earliest pre-embryonic specimens and the next two youngest conceptuses containing fetuses weighing 1.6 g (E58/94; Table 1) and 2.0 g (E86/95; Table 1), and therefore both estimated to be around 4 months of gestation (Craig, 1984). The placental zone was now visible to the naked eye as a narrow, pale, circumferential band, 3 cm wide, from which it was possible to recover full width pieces of the tissue for fixation and sectioning. Histologically, a line of relatively simple frond-like protrusions of endometrial stroma, each invested by trophoblast cells, rose vertically upwards from the surface of the endometrium (Figure 6d, 1.6 g fetus). These lamellate protrusions were beginning to become branched at their tips (Figure 6e, 2.0 g fetus), reminiscent of the early development of the branching microcotyledons on the surface of the equine placenta (Samuel, Allen and Steven, 1974). And at this early stage, an appreciable thickness of endometrial stroma still persisted between the investing trophoblast cells and the endothelium of the maternal blood vessels within the core of each lamella (Figure 6e, 2.0 g fetus). With advancing gestation, however, the stromal lamellae became increasingly elongated, plate like, thinner and more branched, thereby allowing ever closer apposition of fetal trophoblast to the maternal endothelium (Figure 9b, 189 g fetus). Furthermore, at each edge of the placental band, the lengthening placental lamellae began to bend laterally and lay over towards the lumenal surface of the endometrium, thereby forming a blind cleft or cavern between the endometrial and placental surfaces (Figure 7c, d and Figure 9a, 5.15 g fetus; Table 1). The lumenal uterine epithelium within the depths of these lateral clefts became

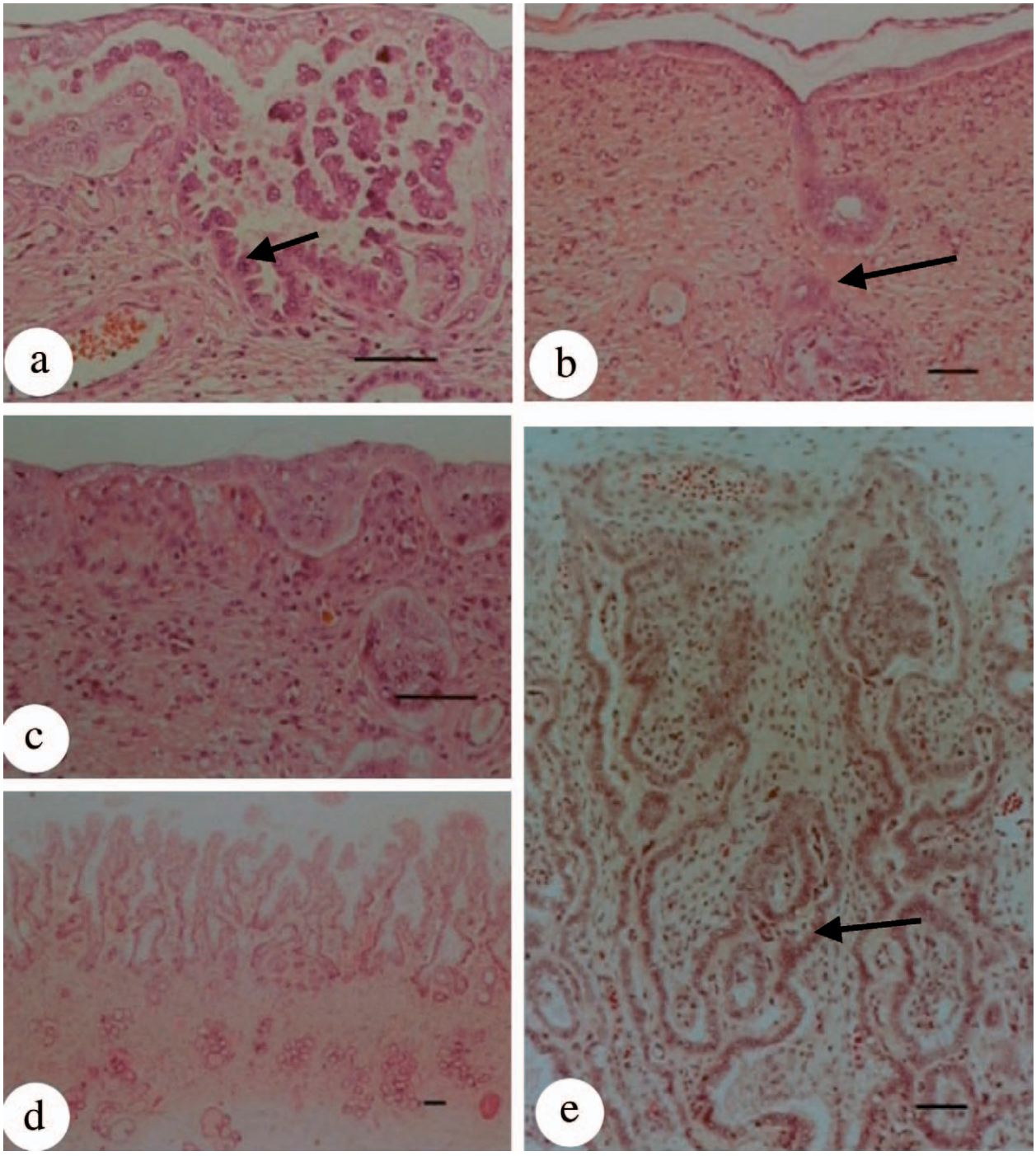

Figure 6. (a) Photomicrograph of the endometrium in elephant E17/93 (no embryo visible; Table 1), showing an elongated finger-like protrusion of trophoblast cells (arrowed) forcing its way beneath the more darkly stained lumenal epithelial cells and lifting them off the basement membrane. Scale bar=90 μm. (b) Photomicrograph of the endometrium in elephant E18/95 (no embryo visible) showing the lumenal epithelium completely replaced by a layer of trophoblast cells that are traversing for a short distance down the mouth of an endometrial gland, lifting the glandular epithelial cells off the basement membrane as they do so (arrowed). However, these invading trophoblast cells do not breach the basement membrane and they fail to enter the endometrial stroma. Scale bar=90 μm. (c) Higher-power photograph of the endometrium in elephant E18/93 (no embryo visible) showing simple blunt upgrowths of endometrial stroma indenting the investing layer of trophoblast. These are likely to be the progenitors of the elongated stromal upgrowths that constitute the maternal component of the later placental band. Scale bar=90 μm. (d) Low power photomicrograph of the developing placental band on a conceptus containing a 1.6 g fetus showing the line of elongating frond-like upgrowths of endometrial stroma, each covered by the single cell layer of trophoblast. Notice the regular, but relatively sparse, distribution of tightly coiled endometrial glands in the stroma beneath. Scale bar=30 μm. (e) Higher power photomicrograph of the elongating and branching trophoblast covered stromal upgrowths seen on a conceptus containing a 2.0 g fetus. At this early stage an appreciable amount of stromal tissue separates the maternal capillaries from the trophoblast cells within each upgrowth although far greater amounts of fetal mesoderm containing fetal blood vessels filled with nucleated fetal red blood cells (arrowed) separate adjacent upgrowths. Scale bar=80 μm.

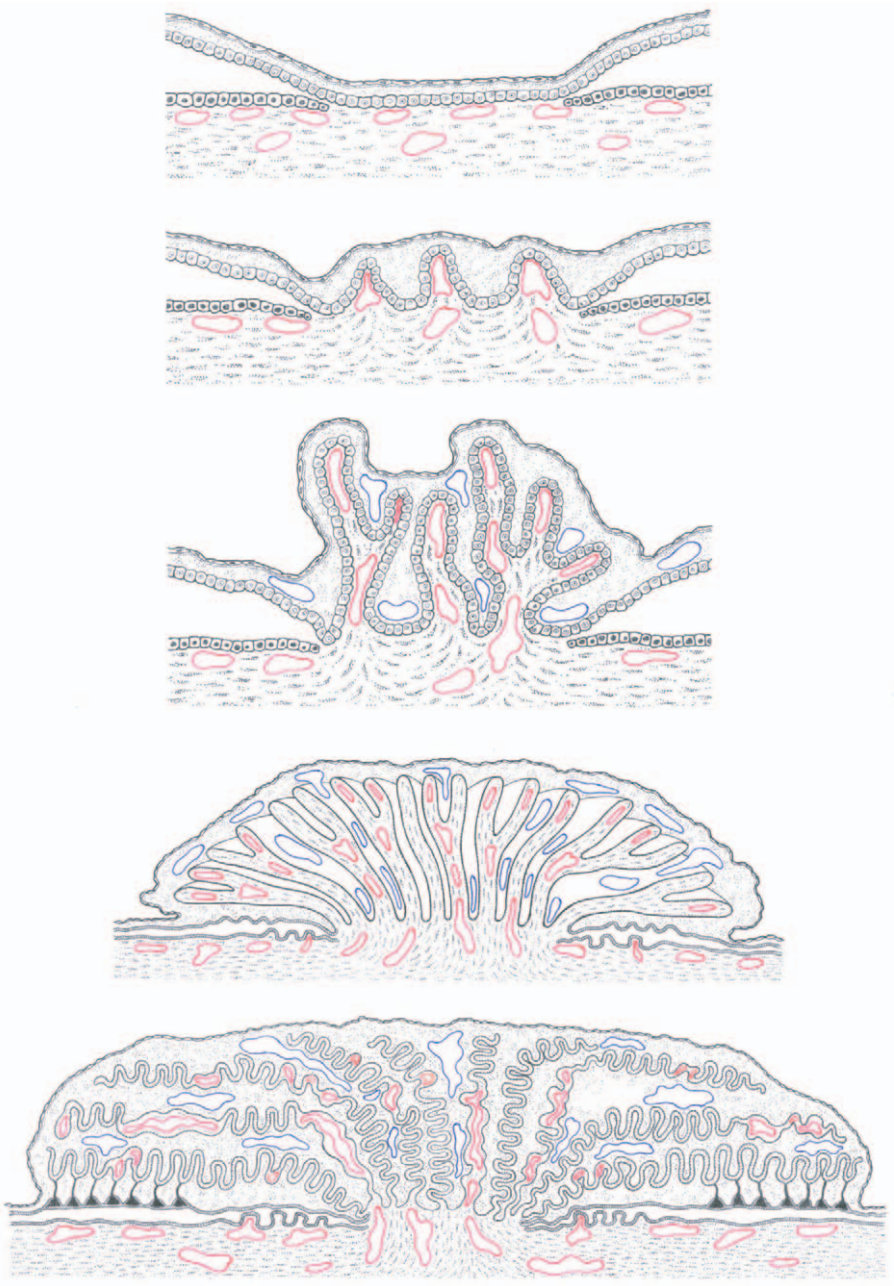

Figure 7. Diagrammatic representation of significant morphological changes that occur during development of the zonary placental band on the elephant conceptus. (a) Replacement of the lumenal epithelium of the endometrium by trophoblast in the equatorial region of the conceptus; (b) Commencing upgrowth of trophoblast covered capillary filled stromal villi above the surface of the endometrium; (c) Elongation and branching of the trophoblast covered lamellae, each containing maternal blood vessels that are still surrounded by adequate amounts of stromal tissue. Fetal blood vessels containing nucleated red blood cells are scattered throughout the mesoderm separating adjacent lamellae; (d) As the branched stromal lamella continue to elongate those at the lateral edge of the band lay over towards the maternal endometrium to create the blind ending cleft in which the haemophagous zone will form; (e) In the mature placental band the now very elongated trophoblast covered stromal lamellae become increasingly folded or pleated so as to maximize the available surface area of fetomaternal contact for placental exchange. Leakage of maternal blood into the lateral clefts of the placental band creates the haemophagus zones in which the morphologically differentiated trophoblast cells take up blood components.

markedly convoluted and the epithelial cells themselves became tall columnar in configuration with blunt pseudopodia protruding from their apical surfaces. In the placental band apposed to the uterine epithelium in the lateral clefts, the trophoblast cells that were bathed in leaked maternal blood in the lacunae in the haemophagous zone had likewise become much taller and flocculent in appearance and could be seen actively taking up the red blood cells that filled the stromal core of the lamellae in this region (Figure 8c and Figure 9d, 17 kg fetus; Table 1).

With advancing gestation, and as seen in sections of the placental zone recovered from conceptuses containing fetuses

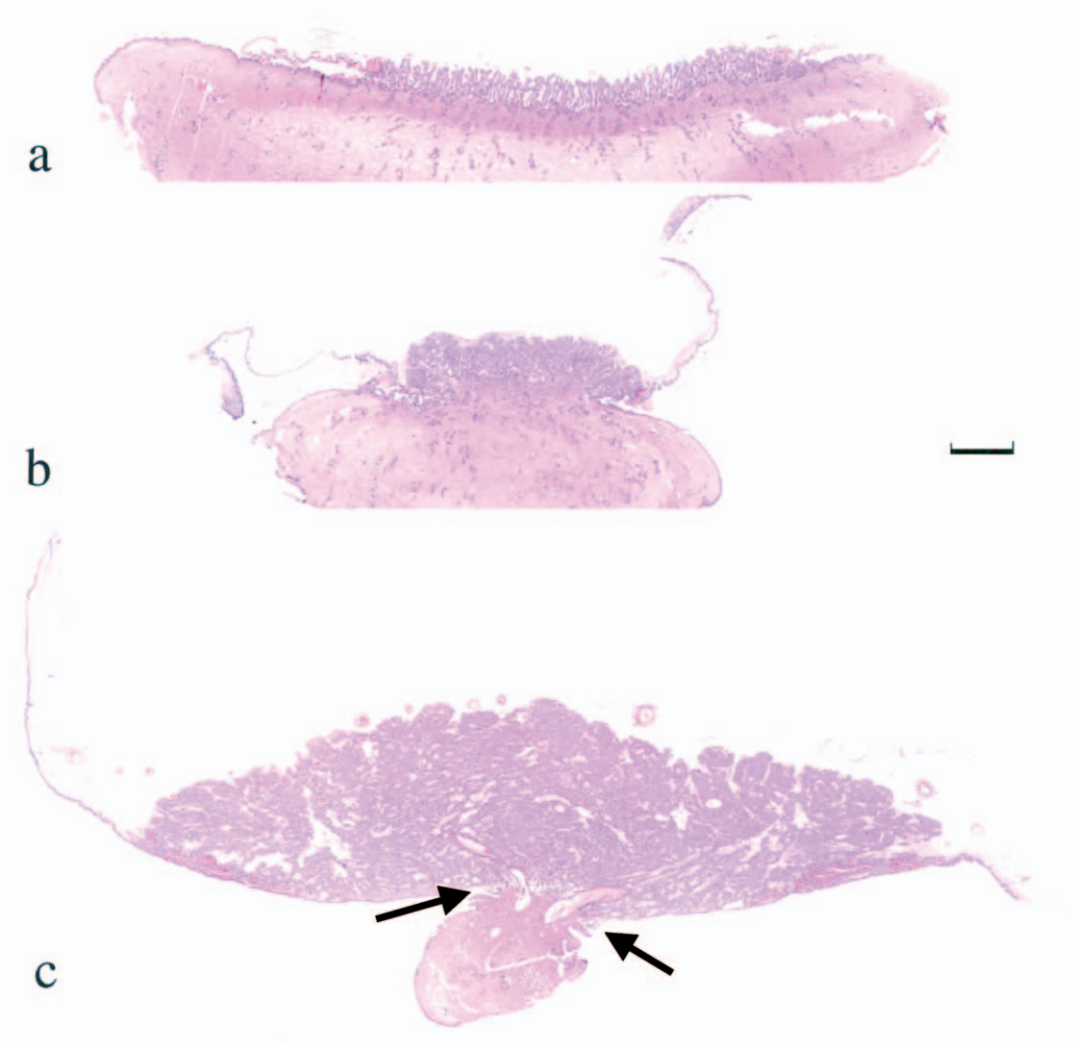

Figure 8. Very low power photomicrographs of the placental band at three distinct stages of development; (a) a conceptus containing a 1.6 g fetus, showing the line of simple trophoblast covered stromal upgrowths above the surface of the endometrium; (b) on a conceptus carrying a 5.15 g fetus, showing elongation and multiple branching of the trophoblast covered stromal lamellae with commencing lateral deviation of the lamellae at the edge of the band; (c) on a conceptus carrying a 120 g fetus, showing marked elongation, lateral deviation and folding or pleating of the trophoblast-covered stromal villi. Note the formation of the haemophagous zones at the lateral edges of the band, the narrowness of the maternal placental hilus that attaches the placental band to the endometrium (arrowed) and the large maternal blood vessels within the hilus. Scale bar=1.7 mm.

weighing 4 g, 20 g, 136 g, 1.9 kg and 17 kg (estimated gestational ages 5–14 months; Laws, 1969; Craig, 1984), the trophoblast covered stromal lamellae containing the maternal vasculature (Figure 7d) increased steadily in length, width and branching complexity, while coincidentally becoming progressively folded or pleated within the confines of the placental band to maximize the surface area of contact between trophoblast and maternal vascular and stromal elements (Figure 7e and Figure 9e, 17 kg fetus; Table 1). The lateral fanning out of the trophoblast covered stromal lamellae was rather akin to the spreading fan-shaped tail of a displaying peacock (Figure 7e and Figure 8c, 120 g fetus) and it showed clearly that the overall size and complexity of the placental interface stemmed solely from elongation, secondary and tertiary branching and finally tight folding of the original stromal upgrowths within their envelope of allantochorion (Figure 7c–e), rather than from any significant increase in either the number or the basic bulk of the primary endometrial protrusions. The width of the maternal placental hilus was not significantly increased after the fetus had grown to 5–10 kg in weight, whereas the overall width and development of the whole placental band increased continuously to term (Figure 5a, 98 kg fetus; Table 1). Within the placental band the trophoblast layer remained cellular with no apparent sign of syncytial formation (Figure 9e). The steady reduction of maternal stroma within each upgrowth continued to bring the trophoblast cells into increasingly intimate contact with the endothelium of the maternal blood vessels (Figure 9b and c), so increasing the efficiency of placental exchange. And in the lateral haemaphagous clefts the leakage and take-up of maternal red blood cells also continued to increase steadily with advancing gestation (Figure 5b, 98 kg fetus and Figure 9d, 1.9 kg fetus).

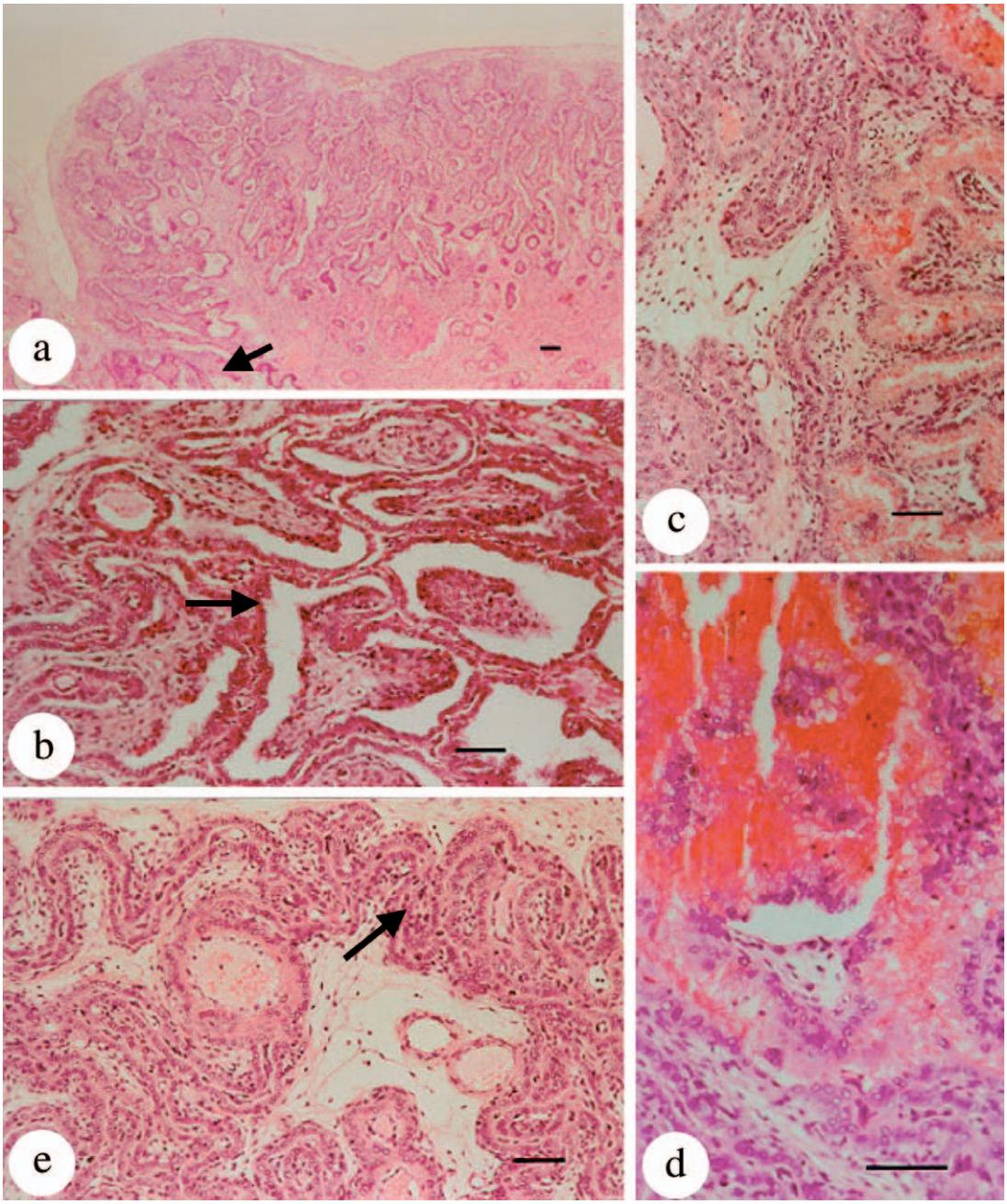

Figure 9. (a) Low power photograph of a transverse section of the placental band on the conceptus carrying the 5.15 g fetus. Note the increasingly branched but still relatively broad, upgrowing trophoblast covered stromal lamellae. At the lateral edge of the band, the lamellae are beginning to lay over towards the surface of the endometrium. Scale bar=30 μm. (b) Higher powered photomicrograph of later placental lamellae on a conceptus containing a 189 g fetus. The maternal stromal component of each elongated and multi-branched lamella is now much thinner and stretched looking, thereby bringing the maternal capillaries and larger blood vessels into more intimate contact with the trophoblast cells (arrowed). The fetal mesodermal component of the lamella is more bulky and prominent. Scale bar=90 μm. (c) Photomicrograph of a transverse section of the placental band on a conceptus containing a 1.9 kg fetus at the edge of the haemophagous zone, showing the uppermost tips of the trophoblast covered maternal stromal lamellae opening out into a series of interconnecting blood filled lacunae. Scale bar=80 μm. (d) High power photomicrograph at the edge of the haemophagous zone on the conceptus containing a 1.9 kg fetus, showing the tall columnar flocculent appearance of the trophoblast cells bathed in and taking up the leaked maternal blood. Scale bar=90 μm. (e) Photomicrograph of a section of the placental band associated with a conceptus containing a 17 kg fetus, showing the pronounced folding or pleating of the trophoblast covered stromal lamellae (arrowed) to maximize the area of feto-maternal contact for placental exchange. Scale bar=80 μm.

Ultrastructural features of placentation

The trophoblast cells that had eliminated and replaced the uterine epithelium at implantation were tall columnar cells with numerous apical mitochondria, occasional phagolysosomes and lipid droplets and a moderate development of rough endoplasmic reticulum (RER) basally (Figure 10a, no embryo visible). They had well developed junctional complexes at their

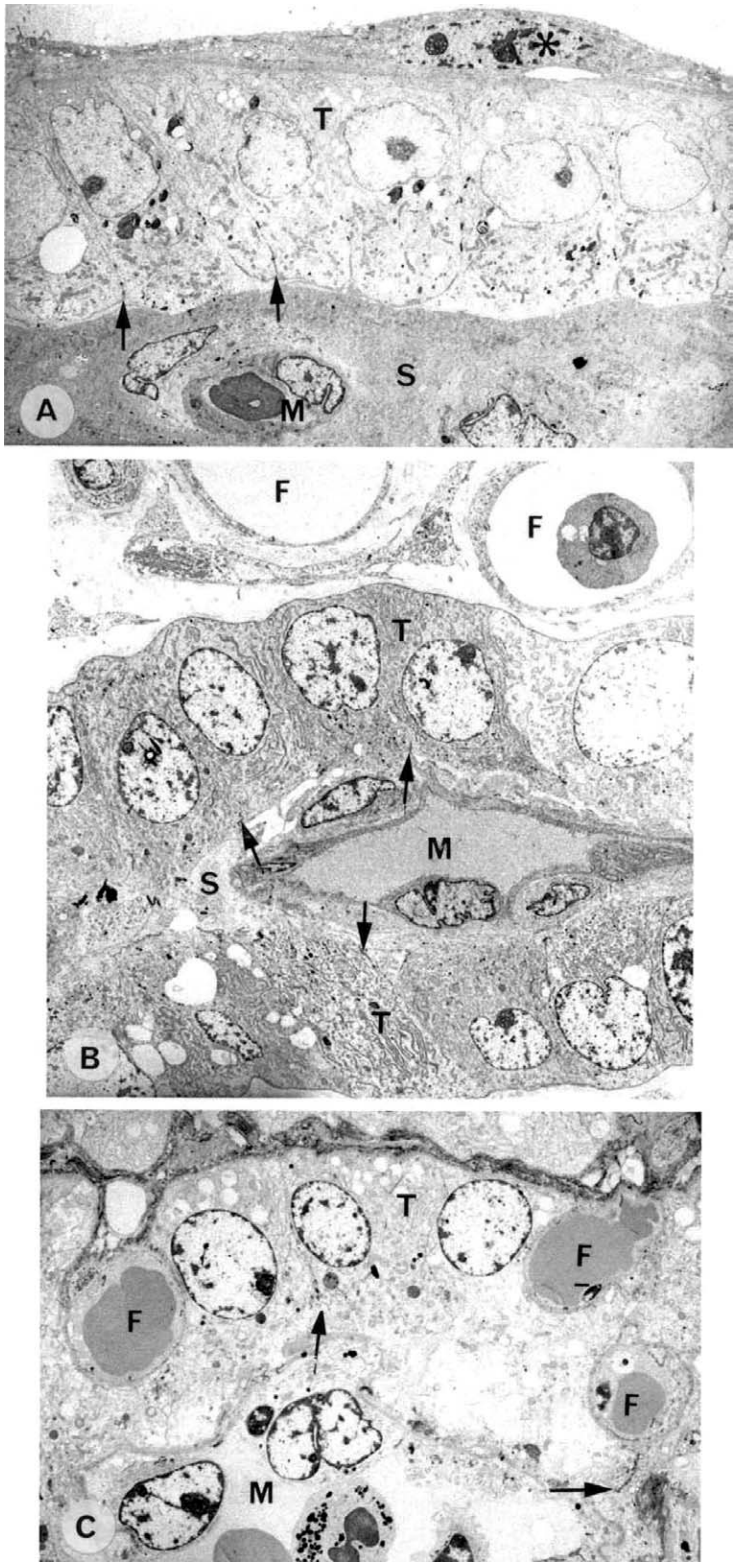

Figure 10. (a) Electron micrograph from the youngest pre-embryonic conceptus (E17, '93 Table 1) showing trophoblast (T) backed by the endoderm (asterisk). The trophoblast has eliminated and replaced the uterine epithelium in the placental band region. Junctional complexes (arrows) seal the apices of the cellular trophoblast which is firmly apposed to the endometrial stroma (S) containing the maternal capillaries (m) (× 2500). (b) Electron micrograph from the placental zone of a conceptus containing a 5.15 g fetus. The fetal capillaries (F) in loose connective tissue are separated from the maternal capillary (M) by tall trophoblast cells (T) sealed at their apices with junctional complexes (arrows). The trophoblast is separated by a thin layer of residual endometrial stroma (S) containing two pericytes (asterisks) (× 2300). (c) Electron micrograph from the placental band of a conceptus containing a 44 kg fetus. The fetal capillaries (F) now deeply indent the cellular trophoblast layer (T), which shows the usual junctional complexes (arrows). This trophoblast is moulded closely around the endothelium of the maternal blood vessel (m) (× 2000).

apices which were firmly apposed to what had originally been the basement membrane of the uterine epithelium. At this early pre-embryonic stage the trophoblast had an underlying layer of flat endodermal cells.

In the conceptus containing a 5.15 g fetus, with well developed placental lamellae (Table 1), the maternal blood vessels were closely invested by the tall columnar trophoblast cells with very little stromal tissue between the two layers (Figure 10b). There was little change in trophoblast ultrastructure at this stage apart, perhaps, from more rough endoplasmic reticulum and fewer phagolysosomes.

In the conceptus containing a 44 kg fetus (Table 1), the trophoblast was still cellular but was very variable in height (Figure 11). As the fetal capillaries now deeply indented the layer they effectively reduced the diffusion distance while coincidentally maintaining the same number of membrane barriers between maternal and fetal circulations. The apex of each trophoblast cell was separated from maternal blood vessels by a considerably thickened basement membrane of the maternal endothelial cells. However, this layer was pierced frequently by narrow processes from the apex of the trophoblast cells which maintained close contact with, but showed no observable junctional specializations to, the endothelial cells (Figure 11). Trophoblast ultrastructure included the normal range of organelles with no obvious specialization to indicate its major transport functions.

DISCUSSION

This study confirmed and extended the earlier findings of Amoroso and Perry (1964) on the development of the elephant placenta and it revealed some other unusual and interesting features of elephant placentation. First, the incredible toughness of the endometrial stroma combined with intense myometrial tone in the non-pregnant animal presumably creates the markedly star-shaped uterine lumen which results in tight apposition of the epithelial surfaces in the lateral branches of the star (Figure 2c). This makes the lumen of elephant uterus more reminiscent of that of the carnivores than of ungulates like the cow and horse in which the endometrium is arranged in longitudinal folds and circular rugae. In their earliest pregnant specimens, where the entire uterus had been fixed by immersion in formaldehyde solution, Amoroso and Perry (1964) noted that the conceptus lodged in one of the lateral branches of the uterine lumen rather than in the central channel. The dissection technique used in the present study enabled sight-directed recovery of fresh tissues for improved fixation but it did not allow confirmation of the site of initial attachment of the young embryo in one of these lateral lumenal branches. The considerable forces applied to the presumably spherical blastocyst as it passes into the uterus from the oviduct will no doubt ensure that it passes down the central core of the uterine lumen until it reaches the implantation site. Quite how this is recognized by the embryo is unknown but it seems to occur always at the point of reflexion of the uterine horn (Perry, 1964, 1974; our own observations). As the embryo begins to expand it presumably opens up one of the star-shaped lateral grooves in the endometrium. The orientation of the embryonic disc may determine the position of the equatorial band of trophoblast cells that interact with the uterine epithelium to initiate development of the placental band (Perry, 1964). It is possible that the positioning of this initial band of interacting cells is determined by internal membrane development independent of outside pressures, with differential uterine growth subsequently ensuring that the placental band is circumferential. Alternatively, it may be the intrauterine pressure that determines the position of the embryonic disc which, in turn, may introduce sufficient asymmetry in the spherical conceptus to give it a precise orientation under certain pressure constraints, thereby ensuring that the placental band will develop circumferentially. Since this initial band of cellular interaction is determined and occurs in the very early stages, interruptions and occasional non-circumferential misalignments of portions the band (Figure 4b) may be explained by the presence of unexpanded grooves and folds in the endometrium.

Lifting and displacement of the lumenal epithelium of the endometrium by protrusions of trophoblast cells, the complete replacement of large areas of lumenal epithelium by trophoblast in the progenitor region of the placental band, and the very beginning of stromal upgrowth, could all be observed in the two very early pre-embryonic conceptuses examined in the present series. The unicellular trophoblast layer protrudes finger-like growths of cells that force their way beneath the maternal epithelial cells and simply lift them off the basement membrane in sheets (Figure 6a). Considering the firm attachment of most epithelia to their basement membrane it seems likely that trophoblast-secreted enzymes may be involved in this process although it could be that the epithelium simply sloughs off the surface of the endometrium in the area of implantation as it does commonly in other species. Whatever the mechanisms involved, the process shows marked similarity to the removal of the lumenal and glandular epithelia of the mare's endometrium by the specialized invasive trophoblast cells of the chorionic girdle region of the fetal membranes to form the gonadotrophin (eCG)-secreting equine endometrial cups (Allen, Hamilton and Moor, 1973). There are, nevertheless, marked differences between the elephant and the horse as to what the trophoblast does once it has lifted the epithelial cells off the basement membrane and bundled them out of the way. In the horse, the binucleate girdle cells eventually pass through the lumenal and glandular basement membranes and stream out into the endometrial stroma where they become tightly packed together to form the endometrial cups (Hamilton, Allen and Moor, 1973). In contrast, in the elephant, the trophoblast cells do not invade the endometrium so deeply and only penetrate into and remove the epithelial cells from the mouths of the endometrial glands (Figure 6a and c). This is also different from the usual process of invasion by trophoblast at implantation in the carnivore endotheliochorial placenta. In the cat (Leiser, 1979; Leiser and Koob, 1993) and

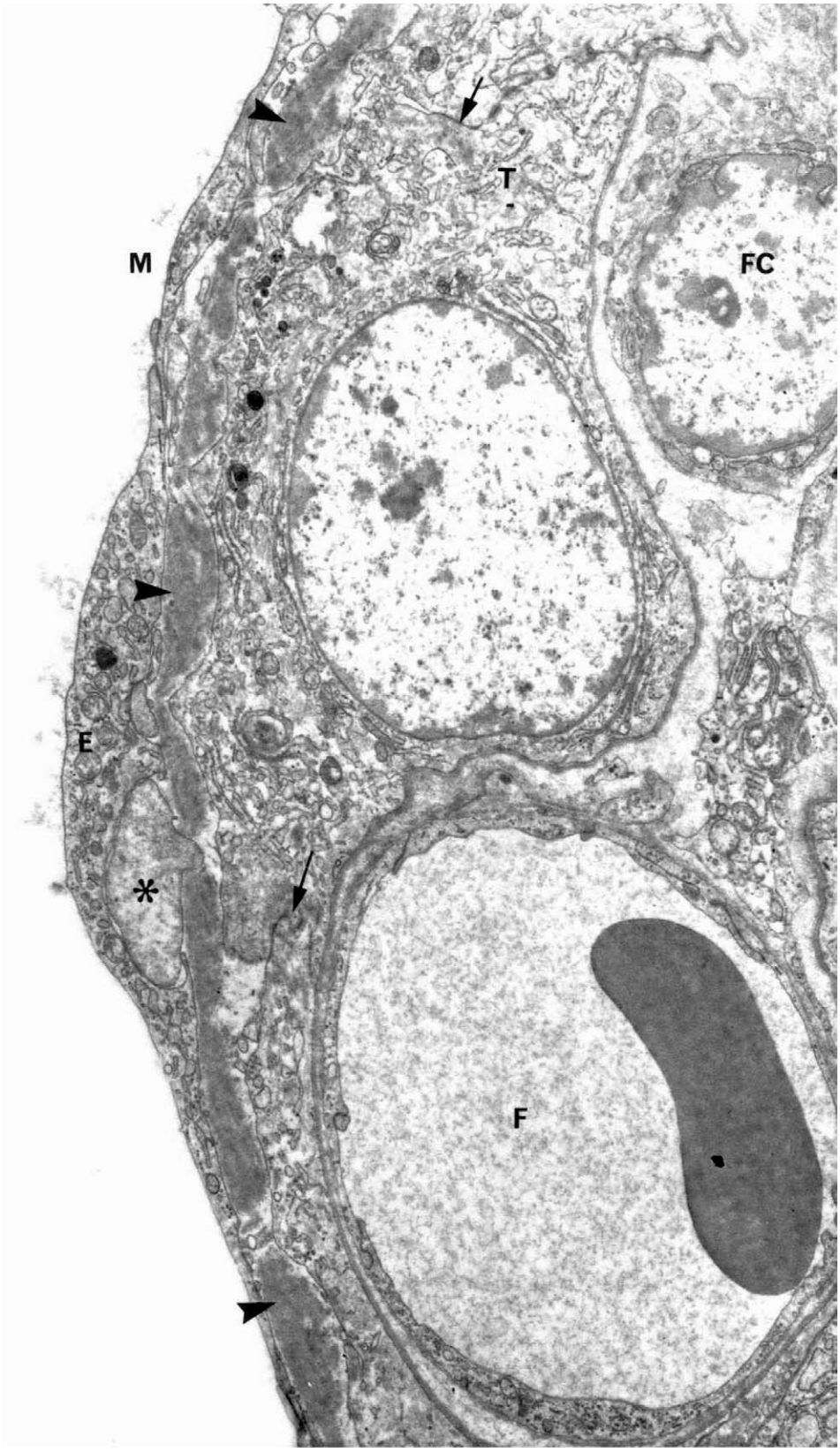

Figure 11. Electron micrograph from the placental band of the conceptus containing a 44 kg fetus. The trophoblast (T) is deeply indented by the fetal capillary (F) which is separated by only <3 μm from the endothelium (E) of the maternal capillary (M). The maternal stroma is reduced to a thickened endothelial basement membrane (small arrows) which is occasionally penetrated by trophoblast processes (asterisk). Serial sections show all such processes are continuous with trophoblast cells. The trophoblast cell boundaries are shown by the long arrows. The fetal stroma contains the occasional fibroblast (FC) but it thins progressively to accommodate the enormous growth of the materno-fetal lamellae (× 8500).

mink (Enders, 1957; Pfarrer et al., 1999), trophoblast contact results in fusion and degeneration of the cells of the lumenal and glandular epithelia and disruption of the basement membrane. The resulting symplasm (Amoroso, 1952) is phagocytosed by the invading syncytial trophoblast which penetrates ever deeper down the glands and into the stroma within the placental band during pregnancy (Leiser and Koob, 1993; Wooding and Flint, 1994).

The lack of invasiveness by the elephant trophoblast may be due to a greater cohesiveness between cells, or a lack of enzymes or receptors necessary to promote deep penetration of the endometrium. The initially subepithelial capillaries and their associated stromal cells are clearly hyperstimulated by the presence of the trophoblast to begin the process of rapid upward growth. Thus, instead of the conventional invasion of fetal tissue into the maternal layer, protrusions of the latter led by the proliferation of the blood vessels, grow up dramatically to supply the demands of an expanding trophoblast and its dependent fetus.

One must assume that the trophoblast cells secrete significant quantities of one or more of the vascular endothelial growth factors (VEGF) family (Ghosh et al., 2000; Charnock-Jones et al., 2001) together with mitogens such as insulin-like growth factor II (IGF-2; Lennard, Stewart and Allen, 1995), hepatocyte growth factor-scatter factor (HGF-SF; Stewart, Lennard and Allen, 1995) and epidermal growth factor (EGF; Lennard et al., 1998). Winther and Dantzer (2001) have demonstrated the presence of VEGF and its receptors in the mink placenta and VEGF seems to be the most likely candidate to initiate the local proliferation of the vascular system which leads to the remarkable growth and development of the placental lamellae in the elephant. It is almost as if the trophoblast draws the endometrial capillaries and stroma upwards in order to create and maximize the total area of feto-maternal contact across which all placental exchange occurs. Furthermore, as the highly vascular stromal upgrowths lengthen they elongate in the plane of the placental band to become plate-like, as well as branching profusely. All of this growth is above the level of the endometrium which, although such development is impressive, it is not unique. It is generally not appreciated that in all types of placenta, no matter how invasive they are initially, around 70–80 per cent of the growth of the materno-fetal interface is above the original plane of the endometrium (Mossman, 1987; Wooding and Flint, 1994). In the elephant, the amount of maternal connective tissue within the upgrowing villus decreases rapidly during the lengthening process, thereby bringing the proliferating trophoblast cells into increasingly intimate contact with the rapidly extending maternal capillary meshwork. These plates of maternal capillaries are bounded on both sides by trophoblast that is backed by rapidly growing fetal capillary networks.

Large arteries passing through the maternal placental hilus run through the full thickness of the placental band at all stages of gestation before the blood drains back through the maternal capillary meshworks to veins in the hilus. Fetal arteries probably do the same in the opposite direction thereby ensuring efficient counter-current flow to maximize transfer potential. However, Leiser and Kohler (1984) showed that, in the endotheliochorial placenta of the cat, the blood flow is cross-current in nature due to a more complex channelling of the fetal blood. Corrosion cast studies of the placental vasculature in the elephant would be required to establish which pattern operates in this species.

The elephant trophoblast is cellular throughout pregnancy with no significant formation of binucleate cells, or of syncytium, at any stage. The maternal and fetal endothelia also persist unchanged throughout pregnancy although the diffusion distance between the maternal and fetal capillaries lessens progressively as the latter increasingly indent the trophoblast, thinning it down to <3 μm in many places. Another change in the second half of gestation in the elephant is an increase in thickness of the basement membrane of the maternal endothelium to form a structure that is equivalent to the amorphous 'intermediate membrane' of the carnivore placenta (Wooding and Flint, 1994).

A striking finding in the present study was the large organizing blood clots in two of the three post-parturient uteri examined and the deep, circular scar in the endometrium of the third animal indicating the permanent effect of repairing the previous placental attachment. Since the elephant placenta develops entirely by stromal upgrowth from the surface of the maternal endometrium, rather than from any penetration of fetal trophoblast into the maternal organ, the final dimensions of that upgrowth must be limited by the relatively small band of maternal and fetal tissues involved in the initial interaction process during implantation. A vast amount of growth, development and architectural modification occurs subsequently above and around that initial band of attachment throughout pregnancy, but the width of the hilus does not increase significantly. Thus, as angiogenesis drives the development of more and bigger blood vessels in the endometrium to service the increasing needs of the growing placenta and fetus, the now relatively narrow maternal placental hilus must become an increasingly concentrated vascular plexus that could be expected to bleed profusely if suddenly ruptured.

Few births of African elephants in the wild have been observed and those that have been monitored (Moss, 1983; Poole, 1996) have involved the parturient cow standing up while she expelled the fetus. The ratio of maternal to newborn weight is very high in the elephant at around 40, compared to a ratio of 15 in the cow and only 8 in the horse (Hammond, 1932). This fact, coupled with the stocky, short-legged frame of the elephant calf, make it likely that passage of the calf through the birth canal during parturition would be rapid and uncomplicated at any stage of gestation. The umbilical cord could not possibly be long enough to enable attachment to persist between both the placenta in utero in the standing parturient mother and the newborn calf ex utero on the ground. Hence, it may be supposed that the cord either ruptures at the umbilicus during the birth process, or it remains intact at this point and the zonary placenta is instead wrenched off the endometrium by rupture of the maternal

placental hilus and its associated vascular plexus. A sudden tearing of this nature would clearly lead to intrauterine haemorrhage and it is tempting to speculate that the large blood clots observed in the two post-parturient cows in this study arose in this manner—a sudden and relatively violent separation of the placental band from the endometrium by the 120 kg mass of the fetus literally falling through the long sloping vagina and out on to the ground.

There is usually little bleeding at delivery in carnivores (Jackson, 1995) in which the endotheliochorial placental band is relatively broader than that of the elephant and there exists a zone of separation just above the myometrial layers which allows release of the pup, often within its membranes and including all the maternofetal lamellae of the complete placental band. Since the maternal blood supply is over a much wider area, each vessel would be relatively smaller and would presumably be shut down more efficiently as part of a gradual process of separation during multilocus delivery at parturition. However, in the elephant, the sudden rupture of the narrow maternal placental hilus may normally cause considerable intrauterine bleeding and the long time needed for the resulting clot to become organized and resorb completely might well be a contributory factor in the 4-year intercalving interval that is commonly recorded in African elephants (Moss, 1983; Poole, 1996). This occurs despite the fact that gestation lasts only half of that time (i.e. 22 months; Laws, 1969; Craig, 1984) and the cow is still lactating heavily when she does finally conceive 4 years after the previous conception. It is possible that parturient intrauterine bleeding is commonplace in the African elephant and acts as a sort of intrauterine device (IUD) which prevents conception during the 2 year post-parturient period. Furthermore, it could be that a series of 'silent' ovulations occurring during this prolonged post-parturient uterine involution and recovery period are the source of the multiple corpora lutea of pregnancy (see Perry, 1953; Hodges, 1998; Allen et al., 2002). These notions and many other fascinating questions remain to be answered before we can better understand the mechanisms involved in conception, development of the placenta, maintenance of the pregnancy state and parturition in the African elephant.

ACKNOWLEDGEMENTS

We are extremely grateful to Dr Ian Whyte, Mrs Colleen Wood and all the other members of the Population Control Unit in Kruger National Park for much practical help in gathering samples and for great kindness and hospitality. We are also grateful to Marlena Ford, Martin Houpt and Domenic Moss for expert technical assistance. John Fuller kindly prepared the diagrams. The Sir Philip Oppenheimer and the Sunley Charitable Trusts gave generous financial support.

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