

Review

Ovulation, pregnancy, placentation and husbandry in the African elephant (*Loxodonta africana*)

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The African elephant reproduces so efficiently in the wild that overpopulation is now a serious problem in some game parks in Zimbabwe, Botswana and South Africa. The female reaches puberty between 10 and 12 years of age in the wild and, when in captivity, shows oestrous cycles of 14–15 weeks duration. She readily conceives a singleton in the wild yet her uterus has the capacity for twins. She shows a gestation length of 22 months and, in the wild, shows a population density and feed dependent intercalving interval of 4–8 years. The trophoblast erodes the luminal epithelium of the endometrium and stimulates upgrowths of blood vessel-containing stromal villi, which develop eventually into the broad, tightly folded lamellae of the zonary, endotheliochorial placenta. Significant quantities of leaked maternal erythrocytes and ferric iron are phagocytosed by specialized trophoblast cells in the haemophagous zones at the lateral edges of the placental band. Although the placenta itself is endocrinologically inert, the foetal gonads, which enlarge greatly during the second half of pregnancy can synthesize 5α -dihydroprogesterone and other 5α pregnane derivatives from cholesterol and pregnenolone. These products may synergize with progestagens secreted by the 2–8 large corpora lutea which are always present in the maternal ovaries throughout gestation to maintain the pregnancy state.

Keywords: african elephant; placenta; foetal gonads; progestagens

1. INTRODUCTION

African elephants are distributed widely over the southern half of the African continent where, it is generally agreed, they exist as two main subspecies; (i) *Loxodonta africana africana*, the most common Savannah or Plains elephant that inhabits the northern, eastern and southern states, from Kenya through Uganda, Tanzania, Zimbabwe, Mozambique, Botswana, Angola to South Africa and also includes the tall and more slender desert elephant, which has adapted wonderfully to survive in the very harsh and arid conditions of northern Namibia; (ii) *Loxodonta africana cyclotis*, the shy and appreciably smaller forest elephant that survives in the dense rainforests of The Congo and other Central and West African states (Roca *et al.* 2001).

Although there is a complete paucity of published data on reproduction in forest elephant, it seems reasonable to postulate that, with the likely exception of the stable equatorial ambient temperature and rainforest food supply of their habitat ensuring a more uniform age at puberty, and intercalving interval, no other significant differences would exist in basic reproductive processes between *L. africana cyclotis* and its bigger and more numerous cousin, *L. africana africana*. Hence, while the data presented in this paper

is derived solely from *L. africana africana*, it is assumed to be pertinent for both subspecies of African elephant.

2. GENERAL REPRODUCTIVE PARAMETERS

Four major *post-mortem* studies of population dynamics and reproductive strategies were undertaken in East Africa during the 1950s and 1960s, when elephant culling was being practised widely to prevent overcrowding and maintain biodiversity in existing game parks. They began with the groundbreaking collection and painstaking dissection of reproductive organs by Dr John Perry, working in Uganda (Perry 1953, 1964, 1974; Amoroso & Perry 1964) and were soon followed by extensive and meticulous surveys carried out by Dr Richard Laws, working with Dr Ian Parker and others, on three separate populations of elephants in Uganda, Kenya and Tanzania (Laws 1966, 1967*a,b*, 1968, 1969*a,b*; Laws & Parker 1968). Around the same time, Dr Ian Buss collected reproductive organs from elephant culled in Uganda (Buss & Savidge 1966; Buss & Smith 1966; Buss & Johnson 1967; Johnson & Buss 1967*a,b*) and Professor Roger Short and his then graduate student, Dr John Hanks, worked on culled elephant in Kenya and Zambia (Short & Buss 1965; Short 1966; Short *et al.* 1967; Hanks 1971, 1972; Hanks & Short 1972).

These pioneering investigations established the benchmarks of reproduction and population dynamics in the African elephant that have been supported and added to since by the long term behavioural

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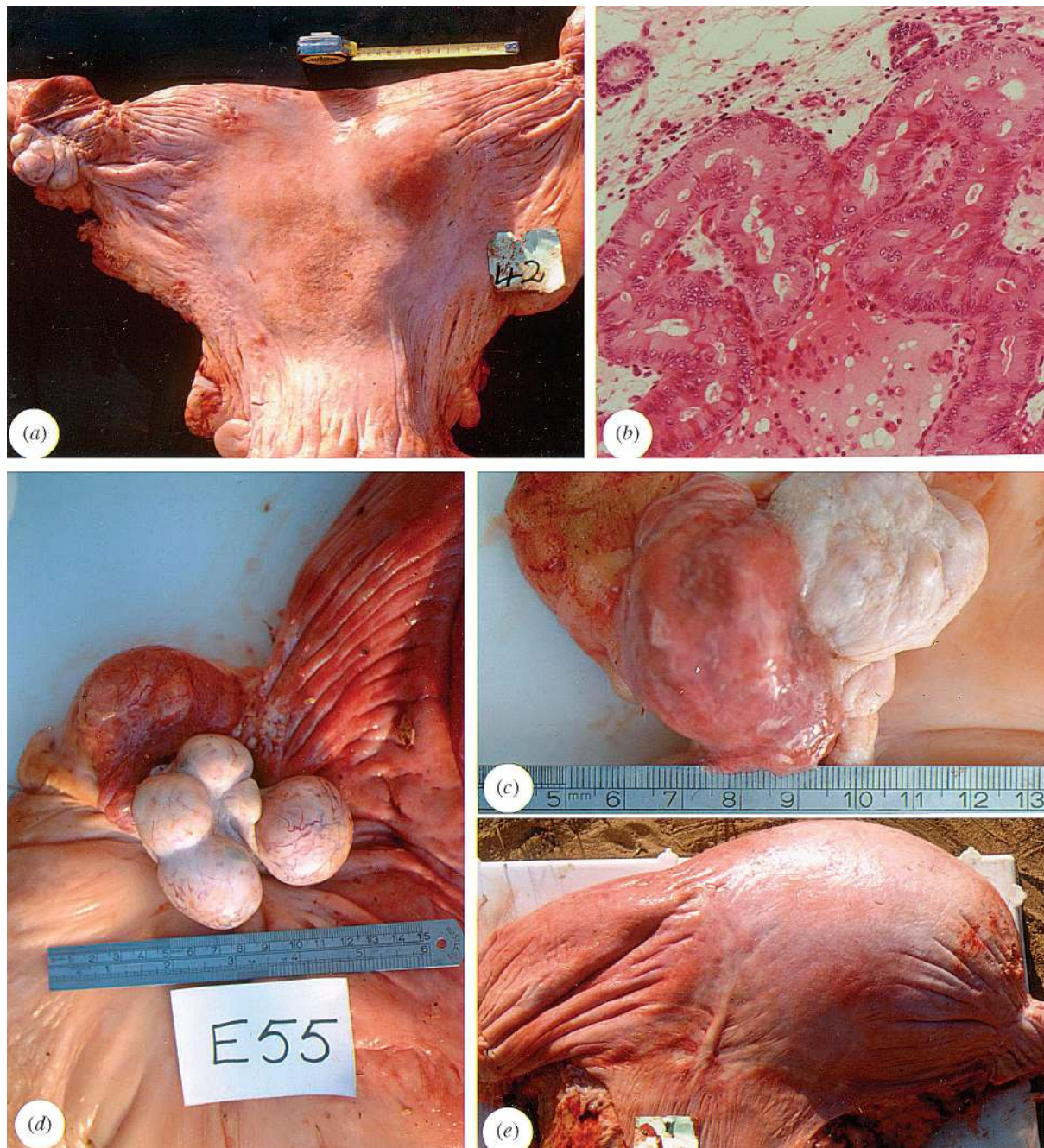


Figure 1. (a) The reproductive tract of a 4.5 month pregnant elephant viewed from the dorsal surface. The discrete conceptus bulge containing a foetus weighing 5.15 g is situated at the lateral divergence of the right uterine horn. (b) Photomicrograph of the endometrium of an adult non-pregnant female elephant showing the very close apposition of the luminal epithelia to almost completely ablate the uterine lumen (Scale bar = 90 μ m). (c) Close-up view of a typical non-pregnant adult ovary extruded from its bursa to reveal the oedematous and velvety mucosal lining of the latter. (d) Four large pregnancy corpora lutea clustered on the ovary ipsilateral to the gravid uterine horn of an elephant at 6.5 months of gestation. (e) Unilateral conceptus bulge containing a foetus weighing 17 kg at 13.5 months of gestation.

observations of Cynthia Moss and Joyce Poole upon the closed, but expanding, herd of elephant in Amboseli Park in southern Kenya (Moss 1983, 1988; Poole 1989, 1996). More recently, Professor J. K. Hodges and colleagues carried out a range of *in vitro* endocrinological investigations utilizing tissues collected from elephant culled in Kruger National Park during the Early 1990s (Hodges *et al.* 1994, 1997; Heistermann *et al.* 1997a,b; Meyer *et al.* 1997; Greyling *et al.* 1997, 1998; Hodges 1998). Simultaneously, a number of valuable physiological and endocrinological observations and measurements have been made on

oestrous cyclicity and pregnancy in captive African and Asian elephants in zoos (Ramsey *et al.* 1981; McNeilly *et al.* 1983; Brannian *et al.* 1988; Plotka *et al.* 1988; Brown *et al.* 1991; Taya *et al.* 1991; Niemuller *et al.* 1993, 1997; Olsen *et al.* 1994; Brown & Lehnhardt 1995; Kapustin *et al.* 1996; Meyer *et al.* 2004) and Professor Raman Sukumar has given a detailed account of the evolution, ecology and conservation problems associated with both Asiatic and African elephants (Sukumar 2003).

In his original *post-mortem* study, Perry (1953) calculated that young African elephants in the wild

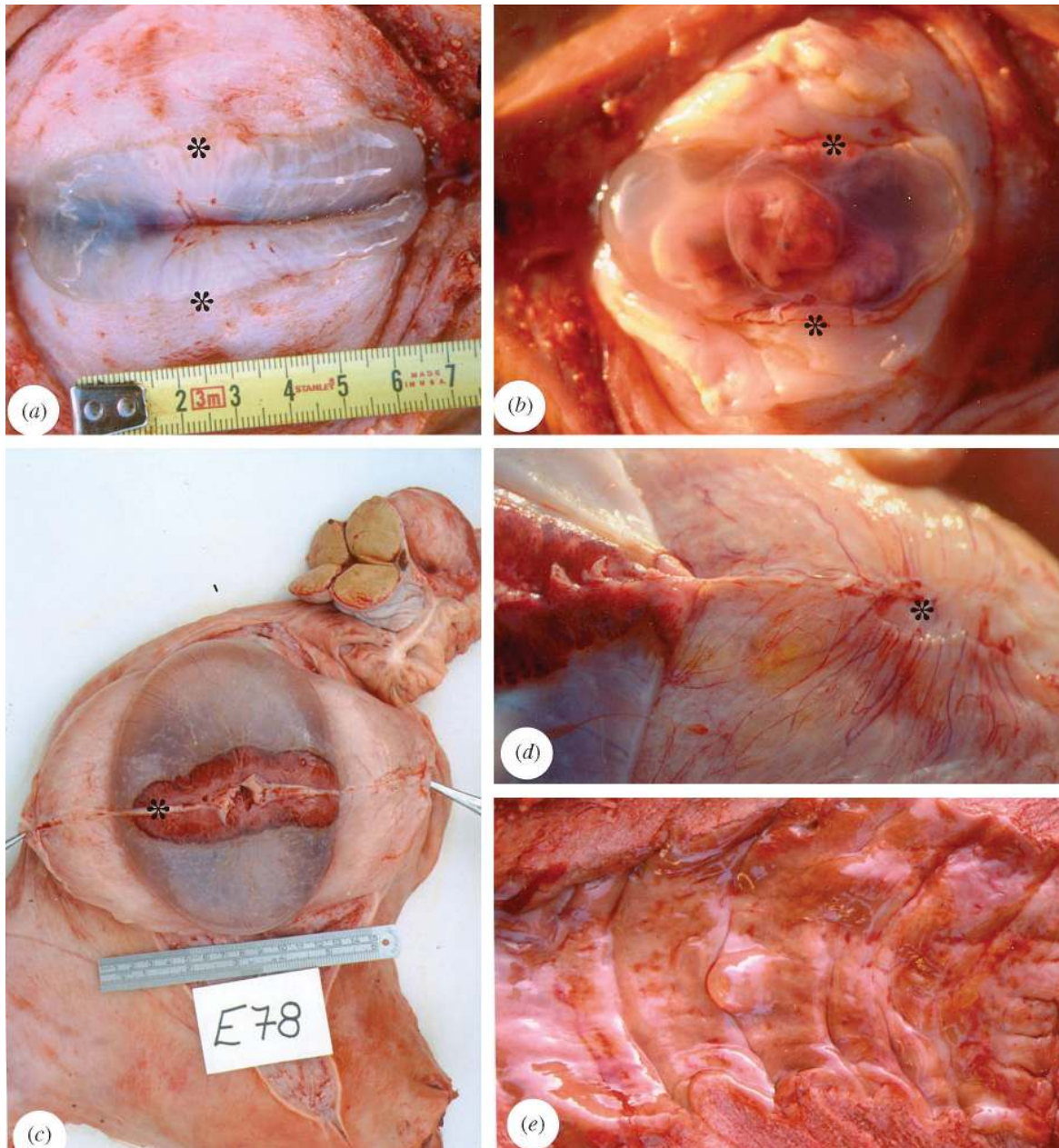


Figure 2. (a) An opened conceptus sac that contained a 1.6 g foetus (four months gestation; Craig 1984). Note the pressurized bulging of the pale, tough endometrium with the attached embryonic membranes. The pale outline of the progenitor placental band is just visible (asterisk), in the middle of which primitive embryonic and placental blood vessels can be seen. (b) A conceptus bulge opened to reveal its 5.15 g foetus at 4.5 months of gestation. The separate allantoic locules can be seen and the transversely sectioned developing placental band is visible at the top of the conceptus (asterisk). (c) The gravid horn at 6.6 months of gestation opened to reveal the ovate conceptus now firmly attached to the endometrium by the thin fibrous-looking maternal placental hilus (asterisk) running in the centre along the length of the thickened and reddish-coloured placental band. Note the khaki-coloured homogenous luteal tissue of the two bisected corpora lutea in the ipsilateral ovary. (d) Close up view of (c) showing the maternal placental hilus being severed from the endometrium to allow the conceptus to roll out of the uterus. Note the relative narrowness of the maternal hilus and the great concentration of endometrial blood vessels feeding into it (asterisk). (e) The endometrial surface of one uterine horn in a very aged (more than 60 years) non-pregnant female, showing five concentric circumferential lines indicating the sites of attachment of the maternal placental hilus in previous pregnancies.

reached sexual maturity between 8 and 12 years of age (mean of 11 years) and males tended to pass through puberty 1–2 years after females. He concluded that mature females were polyoestrous, but showed no evidence of a breeding season, and, under normal conditions, exhibited a mean calving interval of 3.8 years. However, Laws (1969a), studying organs from five separate populations of elephant culled in Uganda, Kenya and Tanzania, with densities ranging from 2.5 to

10 elephant per square mile and experiencing big seasonal differences in rainfall and green feed, reckoned that both the age of sexual maturity and the intercalving interval were very density dependent. His mean age of puberty ranged from 12.5 to 14, and then on to 18, years with increasing population density in the three main populations studied, while the mean intercalving interval varied between 6.8 and 8.9 years. He also noted a definite seasonal pattern of reproduction, with

biannual peaks in conceptions associated with the two rainy seasons, one of which appeared to be retarded in the high-density population in association with nutritional deficiencies. Furthermore, he detected definite 'cycles of recruitment' to the population at intervals of 6–8 years which correlated well with the level of rainfall at the time of the preceding conceptions. He concluded that 'density dependent natural regulatory mechanisms are in operation in elephant populations, which involves changes in reproductive rate'.

Turning to ovarian function, Perry (1953) noticed that the ovaries of sexually mature elephants that were not in lactation anoestrus almost invariably contained more than one corpus luteum (CL). From this he concluded that the elephant was polyoestrous and polyovular. Furthermore, from his finding of multiple luteal structures of varying sizes in the ovaries of pregnant animals, he suggested that 'the corpora lutea (CL) are replaced about mid-pregnancy by a second set which are formed by luteinization of all follicles with antra in both ovaries; some of the larger ones ovulate while many smaller ones do not'. However, Laws (1969a) disputed these interpretations. By serially slicing the ovaries of 109 pregnant females he noted that the actual number of CL ranged from as low as 2 to as high as 42, with a slight downward trend in number during gestation, from around 12 in early pregnancy to 9 near term. By measuring the cross-sectional area of the CL he calculated that the total mean weight of luteal tissue in both ovaries ranged from 19 g in early pregnancy, up to 38 g in mid-pregnancy and then down again to 22 g near term. Thus, Laws (1969a) observed that the amount of luteal tissue in both ovaries remained relatively steady at around 15–40 g throughout gestation and with an almost complete absence of mature-sized follicles (5–7.5 mm diameter) in the ovaries in mid-pregnancy. He concluded that 'the elephant in the wild is seasonally polyoestrous and polyovular, although it produces large numbers of small luteal structures in early pregnancy by luteinization of unovulated follicles'.

Short (1966), on the other hand, had the rare opportunity to *post-mortem* one mature female elephant a few days after she had been seen to be mated. He found one recent CL with an ovulation stigma and three older CL. Having observed as many as seven CL in the ovaries of another non-pregnant elephant he concluded that ... 'the elephant is a polyoestrous monovular animal that undergoes a number of sterile cycles before coming pregnant. These appear to be ovulatory cycles, judging by the appearance of ovulation stigmata, but it is not known if they are accompanied by overt signs of oestrus'. In response, Laws (1969a) summarized the available evidence and suggested that, in the wild, 'the elephant is either: (i) polyoestrous and polyovular, occasionally monovular; (ii) polyoestrous and polyovular, but also produces large numbers of accessory CL by the luteinization of unruptured follicles or (iii) polyoestrous and monovular but forms large numbers of accessory CL'.

Moving on 30 years, reproductive interest in the African elephant in the 1990s concentrated more upon endocrinological aspects of the oestrous cycle and pregnancy. Serial serum progesterone and

gonadotrophin assays carried out during a total of 38 oestrous cycles monitored in adult captive African elephants demonstrated that the whole cycle lasted 13.0–14.1 weeks and was made up of 4–5 weeks of follicular phase followed by 8.5–9.1 weeks of luteal phase. Two distinct surges in serum LH concentrations, three weeks apart, occurred during the follicular or inter-luteal phase, only the second of which resulted in ovulation and the expected rise in serum progesterone levels (Kapustin *et al.* 1996).

Hodges (1998) addressed the longstanding question of whether the elephant is monovular or polyovular. He recalled that the actual number of CL in the ovaries of wild females did not differ markedly between the pre-conception oestrous cycle and pregnancy and, based on the published figures of Smith & Buss (1975), de Villiers *et al.* (1989) and his own group (Heistermann *et al.* 1997a,b; Hodges *et al.* 1997), he suggested that 6–8 'luteal structures' per animal would be a reasonable average. He discounted Short's hypothesis that the multiple CL encountered in pregnant and non-pregnant elephants might reflect accumulation of luteal structures from cycle to cycle in order to achieve a critical mass of productive luteal tissue to support pregnancy. He further argued that no evidence existed for any progressive increase in serum progesterone concentrations during successive non-fertile cycles and nor did serum progesterone concentrations correlate with the mass of CL tissue present in the ovaries (de Villiers *et al.* 1989; Hodges *et al.* 1997). The low incidence of non-pregnant, non-lactating adult female African elephants in the wild (Laws 1969a) and the high conception rates following observed matings (Moss 1983), seemed to argue against a lengthy process of accumulating luteal tissue in sequential 14 week ovarian cycles and Hodges (1998) therefore concluded that the formation of multiple CL, with and without ovulation stigmata, probably occurred in each cycle, with structural, but not functional, persistence into subsequent cycles as the most likely explanation of the visible evidence.

The other major contribution by Hodges and his colleagues in the 1990s was to confirm the original findings of Short & Buss (1965) and Smith *et al.* (1969) of the complete absence of progesterone in the peripheral blood and luteal tissue of cycling and pregnant elephants and its replacement by 5 α -reduced progestagens, especially 5 α dihydroprogesterone (5 α DHP). This important finding, coupled with the experimental observations of Meyer *et al.* (1997) and Greyling *et al.* (1998) that 5 α DHP binds avidly to the endometrial progesterone receptor in the elephant, established the interesting likelihood that, as in the mare and other equids during the second half of gestation (Hamon *et al.* 1991; Holtan *et al.* 1991), 5 α DHP and not progesterone, constitutes the biologically active progestagen which supports the pregnancy state in this species.

In terms of luteotrophic support during pregnancy, McNeilly *et al.* (1983) detected FSH and LH immunoreactivity in peripheral serum samples taken from pregnant wild African elephants at all stages of gestation, but the levels did not differ from those recorded in non-pregnant animals and nor was there

any clear pattern related to gestational age. They, together with Hodges *et al.* (1987) and Brown & Lehnhardt (1995), did however, detect definite increases in serum prolactin concentrations from around 4–6 months of gestation in pregnant African and Asian elephants. Since prolactin was known to be luteotrophic in many other species, Hodges (1998) concluded that it might well perform the same function in the pregnant elephant, although he noted that the sharp rise around 4–6 months might be too late to prolong luteal lifespan beyond that of the cycle. Furthermore, there occurred no marked changes in the number, size or general appearance of the ovarian CL coincident with the prolactin rise. Thus, both the source and the precise role of prolactin during gestation in the elephant remain to be determined.

Most recently, in an excellent study, Meyer *et al.* (2004) measured the profiles of progestagens, prolactin, relaxin and cortisol in serial blood samples recovered throughout pregnancy from 19 Asian and eight African captive elephants maintained at zoos across America. They confirmed the marked rise in serum prolactin levels from around the fifth month of gestation as noted originally by McNeilly *et al.* (1983), recorded a similar rise in serum relaxin concentrations, also from around the fifth month, showed a dramatic rise in serum cortisol concentrations coincident with an equivalent fall in serum progestagen levels during the last 5–8 days before birth and, interestingly, showed a remarkably steady rise in serum progestagen concentrations from the time of ovulation which did not peak until as late as 5 months of gestation. Furthermore, they made the intriguing observation that, between 5 and 20 months of gestation, in Asian but not in African elephants, serum progestagen concentrations were significantly higher in mothers carrying male foetuses than in those carrying female foetuses. Finally, when viewing progestagen profiles in individual females, both Asian and African, there appeared to be a pronounced drop in levels around the end of the first month of gestation, followed by an equally pronounced secondary rise soon afterwards that continued unchecked until peak levels were achieved by month five (Meyer *et al.* 2004). This early gestation profile poses the fascinating question of whether the initial fall and secondary rise in progestagen concentrations around the end of the first month of gestation in the elephant is the result of one or more secondary or accessory ovulations or perhaps reflects a rather sudden enlargement and/or increase in steroid output by the existing multiple CL. And whichever mechanism is in play, what, if any, gonadotrophic stimulus is involved in the process and from where does it originate?

3. ANATOMY OF THE UTERUS AND OVARIES

The anatomy of the elephant reproductive tract was described in detail by Perry (1964), Short (1966), Laws (1969a) and more recently by Allen *et al.* (2002a,b, 2003). Briefly, the uterus is bicornuate, with two relatively long horns that diverge laterally from each other about halfway along their length (figure 1a and figure 5 in the electronic supplementary material). The uterine body is very short and the posterior portions of

the uterine horns are joined to one another by a short intercornuate ligament. In young non-pregnant adult elephants, the endometrium, myometrium and outermost serosa of the uterus are so collectively fibrous, tough and tonic that it is virtually impossible to insert a sharp knife or scalpel blade into the uterine lumen and slit open one horn along its length (see figure 6 in the electronic supplementary material). A transverse section across the whole uterus posterior to the lateral divergence of the two horns shows longitudinal folding of the endometrium to form a star-shaped uterine lumen (see figure 7 in the electronic supplementary material), as described originally by Amoroso & Perry (1964) and Perry (1974). Histological sectioning of the endometrium in this region demonstrates very tight apposition of the luminal epithelia within the lateral clefts of the star (figure 1b), to give a picture more reminiscent of a rodent uterus than an animal as large as the elephant (Mossman 1987).

The ovary of the adult elephant is very small in relation to its body size and it is fibrous and nodular in general appearance (figure 1c), not unlike that of the pig. *In situ* it is completely enveloped by a distinct serosal pouch which has a luscious red mucosa (figure 1c) and which constitutes the infundibulum of the oviduct incorporated in the ovarian bursa (Amoroso & Perry 1964). The mature pre-ovulatory Graafian follicle in the elephant ovary reaches a maximum diameter of only 0.5–1.0 cm (see figure 8 in the electronic supplementary material; Hodges 1998) yet in every one of 58 pregnant uteri examined by the author over a 3 year period, from the very earliest stages of gestation to near term, between two and eight large, plum-like CLs measuring 3–6 cm in diameter (figure 1d, and figure 9 in the electronic supplementary material) were always present, usually, although not invariably, clustered together on the one ovary situated ipsilateral to the gravid uterine horn (Allen *et al.* 2003). When sectioned they exhibited a homogeneous pale brown or khaki-coloured luteal parenchyma with relatively few fibrous trabeculae or other supportive elements (figure 2c). Thus, a marked discrepancy seems to exist between the small size of Graafian follicles in the ovaries of non-pregnant and pregnant elephants and the much larger, multiple CL that are always present in the ovaries of pregnant animals throughout gestation. The question of when and how these very large multiple CL of pregnancy arise remains an intriguing mystery.

4. PLACENTATION

(a) *Gross development of the conceptus*

The elephant foetus exhibits a very slow and flat growth curve (Craig 1984) so that, during the first 40–60 days of gestation, pregnancy can only be 'guessed at' by the presence of multiple large CL in one ovary and a barely discernible conceptus 'bulge' of only 2–3 cm diameter near the anterior-lateral flexion of the ipsilateral horn. Careful dissection down through the very tough uterine stroma is virtually impossible so that the conceptus, under considerable intrauterine pressure, ruptures with a sudden release of a small quantity of embryonic fluid, to leave the thin, pale choriovitelline membrane firmly

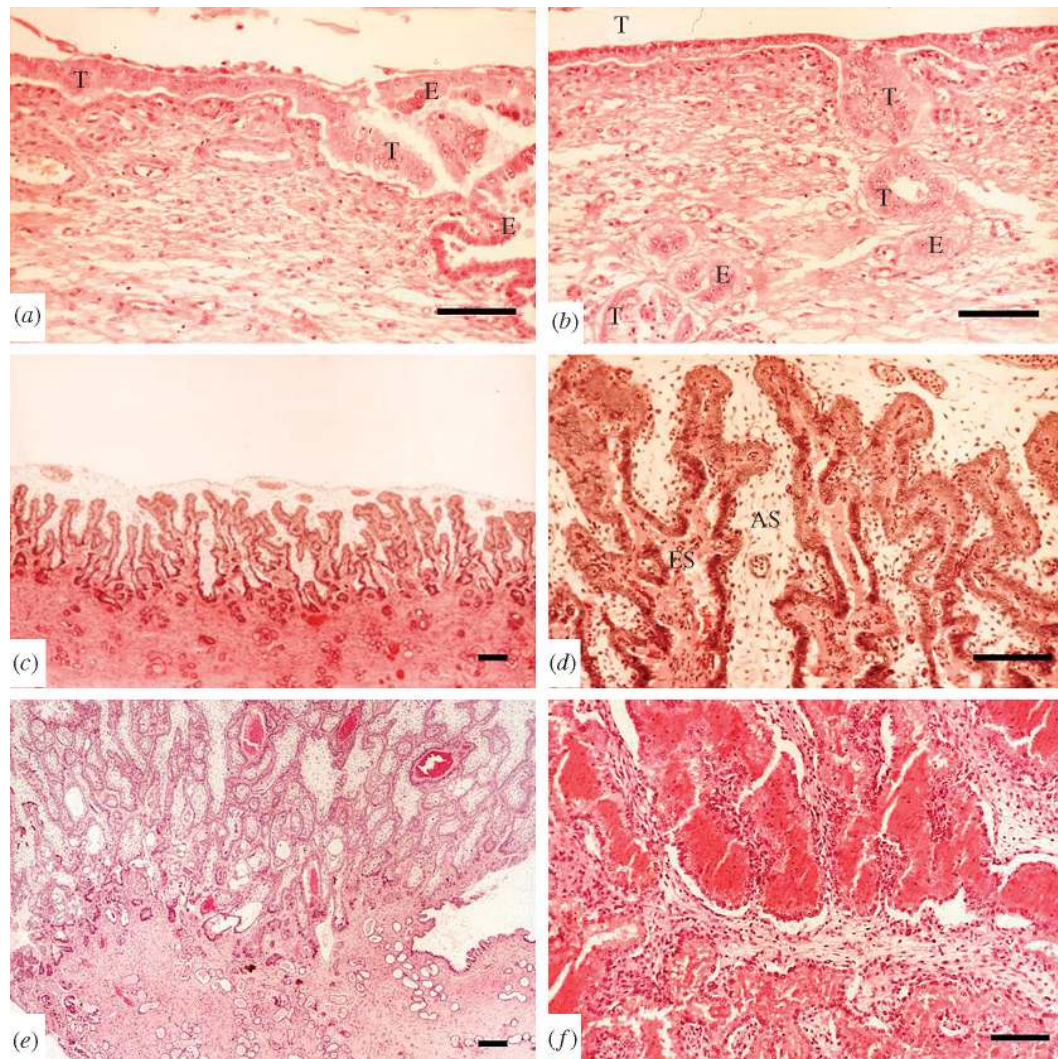


Figure 3. (a) High power photomicrograph of the trophoblast–endometrium interface in the every early stages of gestation (estimated 15–40 days, no embryo visible). A blunt projection of trophoblast cells (T) is forcing its way beneath the more darkly stained luminal epithelial cells (E) and lifting them off the basement membrane (Scale bar=90 μ m). (b) The endometrium–trophoblast interface in a second elephant in the very early stages of gestation (estimated 15–40 days, no embryo visible) showing the trophoblast cells (T) traversing down an endometrial gland by passing beneath and dehiscing the more darkly stained glandular epithelium (E). The basement membrane of the gland remains intact, thereby preventing the trophoblast cells from passing into the endometrial stroma (Scale bar=90 μ m). (c) Low power photomicrograph of the developing placental band on a conceptus containing a 1.6 g foetus at four months gestation showing the line of elongating frond-like upgrowths of endometrial stroma, each covered by the single cell layer of trophoblast (Scale bar=30 μ m). (d) Higher power photomicrograph of (c). At this early stage an appreciable amount of endometrial stroma (ES) separates the maternal capillaries from the trophoblast cells within each upgrowth, although far greater amounts of allantoic stroma (AS) containing the foetal blood vessels separate adjacent upgrowths (Scale bar=90 μ m). (e) Low power photomicrograph at the base of the placental band on a conceptus carrying a 240 g foetus at 6.7 months of gestation. Note the development of the narrow attachment hilus and the continuing upward growth and stretching of the placental lamellae (Scale bar=30 μ m). (f) Section of the placental band on a conceptus carrying a 1.9 kg foetus at 8.85 months gestation at the edge of the haemophagous zone. The specialist columnar trophoblast cells in this region are bathed continuously in extravasated maternal blood (Scale bar=90 μ m).

attached in the equatorial region to the underlying pale and firm endometrium. No embryonic elements are discernible during this very early period. As gestation advances, the conceptus bulge becomes increasingly apparent as a discrete ovate swelling near the lateral reflection of the uterine horn (figure 1a). It then becomes rugby ball-like in appearance (see figure 10 in the electronic supplementary material), moving on to a very large melon that completely fills and extends the gravid horn, but never extends round into the uterine body or non-gravid horn (figure 1c).

This position of the essentially spherical, fluid-filled conceptus in the uterus, which is itself suspended in the

abdomen directly beneath the rectum, makes the pregnant elephant an ideal candidate for transrectal ultrasonography. Dr Thomas Hildebrandt and his colleagues from the Institute of Wildlife Biology in the University of Berlin have, over the past 10 years, successfully performed transrectal ultrasonographic examinations in over 300 Asiatic and African elephants in captivity, using a conventional linear array transducer attached to a ‘rigidly flexible’ extension arm (Hildebrandt *et al.* 1998, 2000a,b; Hermes *et al.* 2004). In addition to making very accurate diagnoses of pregnancy, they have also highlighted a worrying incidence of leiomyomata in the uteri of these captive females (Montali *et al.* 1997).

Pathology of this type, combined with abnormal or absent oestrous cyclicity and a depressing array of behavioural stereotypies, no doubt underlies the very high levels of infertility exhibited by female elephants in captivity (Kurt & Khyne 1996; Schmidt & Khyne 1996; Schmid 1998; Taylor & Poole 1998; Wiese 2000; Brown 2000; Olson & Wiese 2000).

Between 4 and 6 months of gestation, when the foetus weighs 1–5 grams, it is possible to discern the pale, ribbon-like thickening in the equatorial region of the ovoid conceptus that constitutes the developing placental band (figure 2*a–c*) and it is also possible to distinguish the division of the allantois into four separate compartments (figure 2*b*), as described originally by Amoroso & Perry (1964).

The placental band broadens as a result of lateral growth and it becomes increasingly reddish-brown in colour (figure 2*c*, and figures 11 and 12 in the electronic supplementary material). Usually, it forms an unbroken ribbon of tissue around the equatorial region of the conceptus, with uniformly brown-coloured lateral edges indicating the development of the blood-filled haemophagous zones between the placenta and the endometrium (figure 2*c,d*). However, some conceptuses show pronounced narrowing or even breaks of the placental band in some regions (see figure 11 in the electronic supplementary material). In the final stages of gestation, the placental band grows to as much as 12 inches in width and 5–6 inches in depth in the central region (see figures 13–19 in the electronic supplementary material). Its cut surface reveals well-defined areas of homogeneous reddish-brown placental parenchyma separated by fibrous trabeculae. The lateral edges of the band are still much darker brown in colour due to staining by the accumulated maternal blood in the haemophagous zone (see figures 12–19 in the electronic supplementary material; Allen *et al.* 2003).

A surprising feature of the gravid elephant uterus is the relative narrowness of the placental hilus that attaches the conceptus to the endometrium (figure 2*c,d*). The width of this vital connecting bridge is limited to the 1–2 cm breadth of the original upgrowth of stromal villi from the luminal surface of the endometrium during initial development of the placental band and it must carry the plexus of maternal endometrial blood vessels which vascularize the band (figure 2*d*). However, despite the relatively enormous expansion of the placental band throughout gestation, the hilus can gain little additional width and it remains an increasingly narrow channel or tract through which a growing concentration of ever enlarging uterine blood vessels must pass to reach the placental zone. This attachment pedicle can be easily severed using ordinary laboratory scissors which allows the conceptus to simply roll out of the uterus (figure 2*c*; Allen *et al.* 2003).

In recently post parturient uteri a pronounced, trench-like scar can be observed passing around the circumference of the previously gravid horn (see figure 20 in the electronic supplementary material). It clearly indicates the site of attachment of the placental hilus of the previous conceptus and, in the uteri of very aged females, it is possible to discern multiple parallel circumferential scars in the endometrium of both

uterine horns, each indicating the attachment site of previous a pregnancy (figure 2*e*; Laws 1967*b*).

(b) *Histological development of the placenta*

Sometime during the first 10 days after fertilization the spherical embryo enters the uterus and becomes lodged in one of the four lateral clefts that constitute the star-shaped endometrial lumen (Amoroso & Perry 1964). The cuboidal-to-low columnar trophoblast cells of the outermost choriovitelline membrane erode and replace the luminal epithelium of the endometrium over a considerable area of the chorion (figures 3*a* and 4*a*; Amoroso & Perry 1964; Allen *et al.* 2003). Blunt outgrowths or elongated fingers of trophoblast force their way beneath the luminal epithelium and simply lift it off the basement membrane in sheets (figure 3*a*, and figure 14 in the electronic supplementary material). They traverse a short distance down the endometrial glands, again by lifting the glandular epithelial cells off their basement membranes, which they seem unable to cross (figure 3*b*, and figure 14 in the electronic supplementary material). In one small, well-defined centrally located area, simple pointed upgrowths of endometrial stroma filled with maternal capillaries are seen to indent the investing trophoblast layer (figure 4*b*, and figures 15 and 16 in the electronic supplementary material). They are clearly the progenitors of the elongated stromal plates that will eventually comprise the parenchyma of the placental band and their upward growth from the surface of the endometrium gives the strong impression of a stromal response to the mitogenic, and possibly also motogenic, actions of one or more growth factors being secreted by the stromal tissue itself and/or the investing trophoblast cells (Allen *et al.* 2003).

By 3–6 months of gestation the placental zone is now visible to the naked eye as a pale, circumferential band, approximately 1–2 cm wide, passing around the equatorial region of the still spherical conceptus (figure 2*b*). The upgrowths of trophoblast-invested endometrial stroma have now grown into a discrete line of simple frond-like protrusions of stroma, each still closely invested by its layer of trophoblast cells and all rising vertically upwards from the endometrial surface somewhat like a closely planted bed of new asparagus shoots (figure 3*c*). They begin to branch at their tips (figure 3*d*) and, at this early stage, an appreciable amount of endometrial stroma still persists between the investing trophoblast cells and the endothelium of the maternal blood vessels contained within the core of each villus (figure 4*c*). With advancing gestation, the stromal lamellae become thinner and more stretched in appearance thereby allowing ever closer apposition of foetal trophoblast to maternal endothelium (figures 3*e* (also figure 18 in the electronic supplementary material) and 4*d*). Coincidentally, the lengthening placental lamellae at the edge of the placental band lay over laterally towards the endometrium to form a blind cleft between the endometrial and placental surfaces (figure 4*d*, and figure 17 in the electronic supplementary material). The luminal epithelium within these lateral clefts becomes convoluted and the epithelial cells themselves assume a tall columnar configuration with blunt pseudopodia protruding from their apical

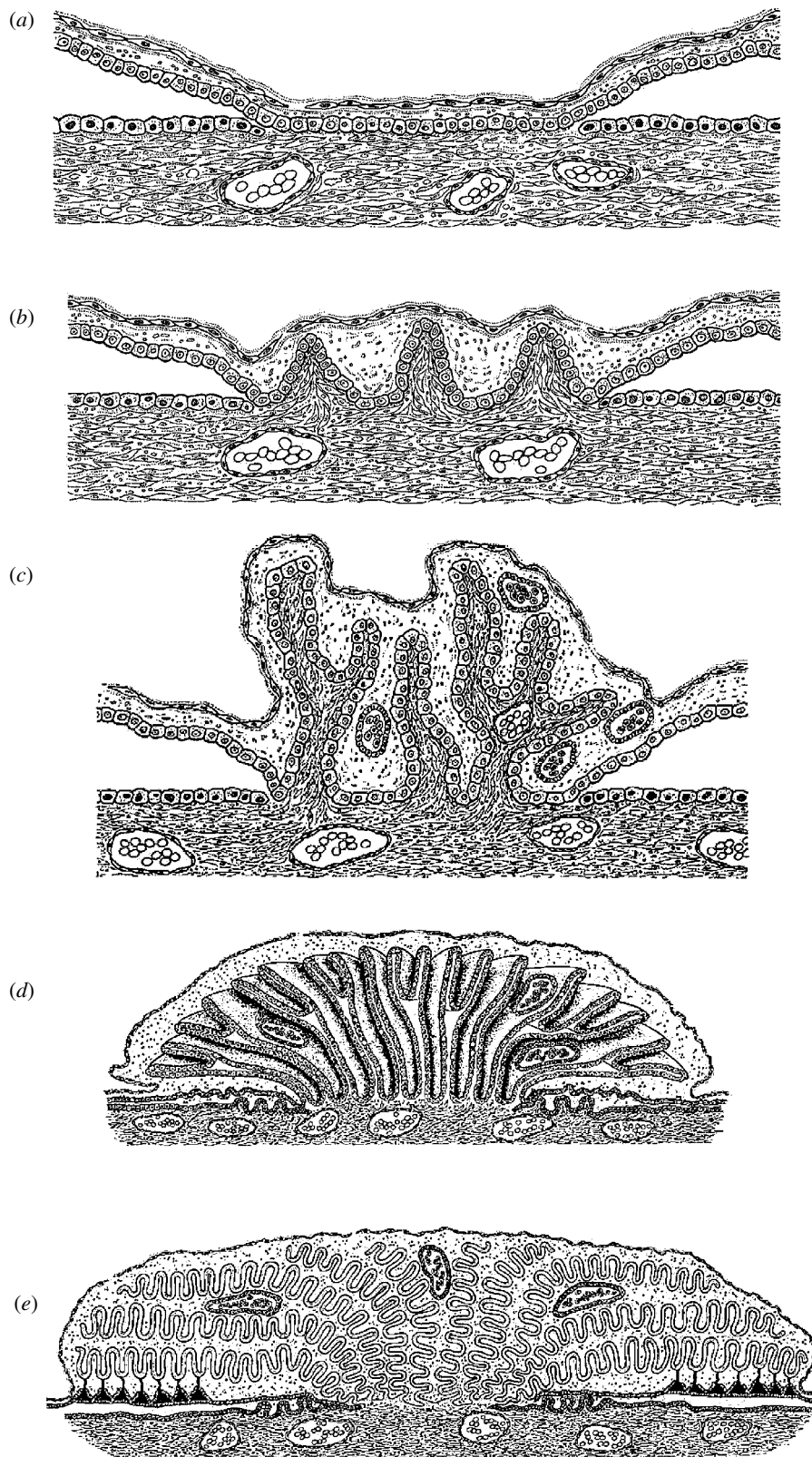


Figure 4. Diagrammatic representation of significant morphological changes that occur during the development of the zonary placental band on the elephant conceptus. (a) Replacement of the luminal epithelium of the endometrium by trophoblast in the equatorial region of the conceptus. (b) Commencing upgrowth of trophoblast-covered stromal villi above the surface of the endometrium. (c) Elongation and branching of the trophoblast covered lamellae, each containing maternal capillaries surrounded by adequate amounts of stromal tissue. Foetal capillaries containing nucleated red blood cells are scattered throughout the allantoic mesoderm that separates adjacent lamellae. (d) The elongating lamellae at the lateral edge of the placental band lay over towards the endometrium to create the blind ending cleft in which the haemophagous zone will form. (e) In the mature placental band, the very elongated 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 creates the haemophagous zones in which the morphologically differentiated trophoblast cells imbibe maternal blood components.

surfaces. Erosions of the endothelium of the maternal vessels in the region cause leakage of maternal blood into both the clefts and the core of the placental lamella (figure 4d). The trophoblast cells bathed in the extravasated blood likewise become taller and flocculent in appearance and they actively phagocytose the red blood cells and other blood components (figure 3f).

With advancing gestation the placental lamellae increase steadily in length, breadth and branching complexity, while coincidentally becoming progressively folded or pleated within the confines of the placental band, to maximize the surface area contact between trophoblast and maternal vasculature (figure 4e). The trophoblast comes into ever more intimate contact with the endothelium of the maternal vessels and these and their foetal equivalents, indent deeply into the trophoblast layer to reduce the interhaemal distance between the foetal and maternal circulations to only 2–3 µm (Wooding *et al.* 2005).

The lateral spreading of the placental lamellae shows that the increasing size, complexity and exchange area of the placental interface stems solely from elongation, secondary and tertiary branching and, finally, tight folding of the original stromal upgrowths within their envelope of allantochorion rather than from any significant increase in either the number or the basic bulk of the primary endometrial protrusions (Allen *et al.* 2003). The width of the placental hilus attaching the conceptus to the endometrium hardly increases at all and, within the placental band, the trophoblast layer remains unicellular throughout, with no sign whatever of syncytial formation (Wooding *et al.* 2005).

(c) Placental endocrinology

As in *Equidae* (Cole *et al.* 1933; Amoroso & Rowlands 1951; Hay & Allen 1975), the gonads of the elephant foetus, both male and female, undergo considerable enlargement during the second half of gestation (figures 21–23 in the electronic supplementary material; Perry 1964; Hanks 1971; Allen *et al.* 2002a, 2005). Histologically, the fibroblast-like interstitial cells in the gonadal stroma undergo hypertrophy and hyperplasia from about the tenth month of gestation and they clump together to form 'blocks' or wedges of epithelioid-type cells between the fibrous supporting trabeculae in the foetal testis (figure 24 in the electronic supplementary material) and coronae of cells surrounding partially developed antral follicles in the foetal ovary (figure 25 in the electronic supplementary material; Allen *et al.* 2002a). The foetal ovaries become appreciably bigger than the foetal testes between 14 and 18 months of gestation due to the partial growth and antrum formation in follicles in the former, but they even up again in size and weight during the final months as the follicles regress again and become atretic (Allen *et al.* 2005).

In the maternal ovaries, 2–8 large plum-like CL, usually clustered on the one ovary ipsilateral to the gravid uterine horn (figure 1d), appear in the very early stages of pregnancy and persist throughout gestation (Smith & Buss 1975; Hodges *et al.* 1997; Hodges 1998; Allen *et al.* 2002a). These pregnancy CL are much larger than the maximum diameter of a preovulatory follicle (Allen *et al.* 2003) and their homogeneous luteal tissue contains high

concentrations of 5 α -dihydroprogesterone and other 5 α -pregnane derivatives, but negligible amounts of native progesterone (Smith *et al.* 1969; Hodges *et al.* 1997).

The sudden appearance and persistence of multiple large CL in the maternal ovaries during pregnancy indicates the possibility of the production of some sort of placental gonadotrophin in the elephant. Furthermore, the enlargement of the foetal gonads during the second half of gestation in the elephant, which parallels similar foetal gonadal hypertrophy in equids (Cole *et al.* 1933; Hay & Allen 1975), likewise suggests the involvement of a gonadotrophin of possibly placental origin. It also indicates the possibility of fetoplacental cooperation in the synthesis of oestrogens, as demonstrated in the mare (Bhavnani *et al.* 1969, 1971; Bhavnani & Short 1973a,b; Cox 1975; Tait *et al.* 1983, 1985) and in women (Diczfalusy 1964). However, closer investigation has so far failed to support either possibility.

Minced placenta collected freshly from 11 elephants carrying fetuses ranging in gestational age from 5.5 to 20.6 months (Craig 1984) and incubated *in vitro* with ³H cholesterol, ³H pregnenolone and ³H androstenedione, failed to synthesize any progestagens, androgens or oestrogens. However, similar incubation of minced gonad recovered from six fetuses aged 13–21 months, but not from three younger fetuses, with ³H cholesterol or ³H pregnenolone, resulted in appreciable conversion of the labelled precursors to 5 α -dihydroprogesterone and other 5 α -reduced progestagens (Allen *et al.* 2002a). This was supported by strong immunohistochemical labelling of the interstitial cells of the enlarged gonads with an antibody specific for 3 β hydroxysteroid dehydrogenase, the enzyme responsible for converting pregnenolone to progesterone and other progestagens (figure 26 in the electronic supplementary material). In parallel experiments, saline homogenates of fresh placenta recovered from five conceptuses aged 4.5–11.0 months (Craig 1984) submitted to a mouse oocyte recovery rate bioassay for total gonadotrophins (Adams 1982), a pig testis radioreceptor assay for FSH (Maghuin-Rogister *et al.* 1978) and an amplified enzyme-linked immunoassay for equine eCG/LH (Allen *et al.* 2002b), all failed to show any hint of gonadotrophic activity (Allen *et al.* 2002a).

Thus, like the zovary placentae of felids and canids, it seems highly unlikely that the elephant placenta secretes any sort of gonadotrophic hormone and the source and timing of the gonadotrophic and/or luteotrophic stimuli which result in the multiple CL of pregnancy and the enlargement and steroidogenic activity of the foetal gonads remains an intriguing mystery. The rise in serum prolactin levels that has been reported to occur in elephants between the fourth and sixth months of gestation (Hodges *et al.* 1987; Meyer *et al.* 2004) might well supply luteotrophic support to ensure persistence of the CL throughout gestation. But it is unlikely to be involved in the initial formation of these luteal structures or in the enlargement and steroidogenic activity of the foetal gonads.

(d) Placental transport:

The two glucose transporter proteins, GLUT-1 and GLUT-3 are present on the trophoblast cells of the

elephant placenta throughout gestation, with GLUT-1 immunoreactivity confined to the basolateral surfaces of the cells and to the internal and outer tissue surfaces of the placental blood vessels. GLUT-3 staining, on the other hand, occurs at the apex of the trophoblast cells, in the flat apical plasmalemma apposed to the maternal basement membrane derivative of the maternal capillaries (Wooding *et al.* 2005). Ferric iron is localized to only the basement membrane of the specialized absorptive trophoblast cells in the haemophagous zone of the placenta which is perhaps not surprising in view of the observed phagocytosis of large numbers of maternal red blood cells by these cells. This uptake is no doubt a very important source of iron, and perhaps other heavy metals and vitamins, for the growing foetus (Wooding *et al.* 2005).

5. DISCUSSION

The African elephant (*Loxodonta africana*) is easily the largest of the world's land-based mammals yet, curiously, embryological evidence of functional nephrostomes persisting during kidney development *in utero* (Gaeth *et al.* 1999), the existence of the elongated and highly mobile proboscis or trunk and the absence of a pleural cavity (Short 1962), all point to a relatively recent evolutionary emergence of the elephant from an aquatic ancestry (Gaeth *et al.* 1999).

The many well intentioned and well executed *post-mortem* studies performed on culled elephant in Africa over the past half century, combined with more recent endocrinological investigations carried out on living animals in zoos and small game parks, have established the basic parameters of reproduction in *L. africana*. The female passes through puberty between 10 and 18 years of age depending upon population density and nutritional availability (Perry 1953; Laws 1969a), she should show regular oestrous cycles of 14–15 weeks duration when unmated (Kapustin *et al.* 1996), she conceives readily a singleton pregnancy from the two or three matings she undergoes by the musth bull when she is in oestrus (Poole 1996), but she very rarely conceives twins, despite adequate room in her uterus to gestate twin conceptuses.

The young elephant embryo develops a zonary endotheliochorial placenta, first by eroding the luminal epithelium of the endometrium and replacing it by trophoblast and then by inducing the underlying endometrial stroma and blood vessels to commence an ever increasing upward growth above the surface of the endometrium to form the highly crimped and folded lamellae that provide steadily increasing placental exchange throughout the 22 months of gestation. Typical haemophagous zones develop beneath the lateral edges of the placental band in which leaked maternal erythrocytes, ferric iron and no doubt many other essential metals, vitamins and minerals are actively taken up by specialized trophoblast cells in the region. And in the centre of the ever widening placental band, a remarkably narrow hilus, through which all the endometrial blood vessels traverse to vascularize the placenta, attaches the whole conceptus to the endometrium. Severance of this attachment pedicle at term, during or soon after calving, is

associated with considerable intrauterine haemorrhage which may contribute to the 4 year or greater inter-calving interval exhibited by most African elephants (Laws 1969a; Moss 1983; Allen *et al.* 2003).

Although architecturally straightforward and apparently hormonally inert (Allen *et al.* 2002a), the elephant placenta nonetheless leaves a number of puzzling questions concerning its wider role in both the induction and maintenance of the pregnancy state. For example, when and how do the large CL of pregnancy develop, why are they so much bigger and more numerous than the ovarian follicles from which they presumably originate, is there one or more secondary or accessory ovulations around the end of the first month of gestation as the recent results of Meyer *et al.* (2004) would seem to suggest, and do these pregnancy CL, either primary or secondary, derive any gonadotrophic or lutetrophic support from the placenta? Does the 5 α DHP and other progestagens secreted by the enlarged foetal gonads during the second half of pregnancy add significantly to those secreted by the large pregnancy CL in terms of maintenance of the pregnancy state and what, if any, is the nature and source of the gonadotrophic stimulus which drives this foetal gonadal enlargement? Could it stem from activity of the foetal pituitary gland during the second half of gestation or might the increased rate of secretion of prolactin from around the fifth month of gestation (Hodges *et al.* 1987; Meyer *et al.* 2004), from the maternal pituitary gland or perhaps even the placenta, act in a quasi-gonadotrophic manner to bring about the observed changes? There is much yet to be learned about the physiology of pregnancy in this magnificent species.

With the high level of fertility existing in both the large mating bulls and the oestrous cows leading to high conception and pregnancy rates (Moss 1988), the 22 month gestation period in which early and late pregnancy losses seem to occur infrequently (Laws 1969a; Moss 1983, 1988), and a high postnatal survival rate combined with general longevity (Moss 1988), it seems that the African elephant, if not killed by its only predator, man, has the inherent fertility mechanisms to increase its population size by a remarkable 8% *per annum* (Whyte 2001). Inevitably, this high reproductive rate leads to overpopulation in secure and well-managed game parks with the accompanying remorseless loss of biodiversity as the elephants destroy the habitat, together with the risk of horrendous mass die-offs in times of drought. Yet, coincidentally and sadly, with the burgeoning human population and associated demand for arable land in other parts of Central and Eastern Africa, combined with the ever present pressure of illegal poaching in these less closely policed areas, whole populations of elephants are becoming increasingly endangered and could even face complete extinction in coming years (Sukumar 2003).

The resulting paradox of 'too many here and too few there' is not easily solvable by seemingly simple procedures, such as physical translocation of whole families of elephant or the creation of migratory corridors between overstocked and understocked parks. For example, darting and extensive translocation

of almost 1000 elephant from Kruger National Park over the past decade has completely filled any requirements for elephant in smaller private game reserves throughout South Africa and neighbouring countries (Whyte 2003). Similarly, a recent attempt to remove much of the elephant-proof fence between Kruger Park and the neighbouring very understocked Limpopo National Park in Mozambique, followed by physical translocation of whole families of Kruger elephant into Limpopo Park, resulted in remarkably rapid migration of the elephant along the persisting fence and back to their home range in Kruger Park (Ian Whyte 2004, personal communication).

Since routine annual management culling of elephant ceased in Kruger Park a decade ago in 1995, the total elephant population in that park has risen from 8000 to just over 14 000 (Rogers 2005). And in Hwange Park in northern Zimbabwe, where management culling ceased in 1985 and in the adjoining and contiguous Chobe National Park in neighbouring Botswana, where elephant culling has never been practised, the last aerial census in 2001 estimated a total of 140 000 elephant in the two parks, at a density of 2.9 elephant for km², which greatly exceeds the widely accepted sustainability norm of 0.5 elephant for 1 km² (Foggin 2003; WWF-SARPO Report 2003; D. Gibson 2004, personal communication). Thus, the timebomb of elephant overpopulation exists in these two lovely game parks at an alarming level and a desperate and urgent need exists to re-commence organized professional culling in all three parks, Hwange, Chobe and Kruger, to preserve habitat, reap the benefits of commercial utilization of the elephant products (meat, skin and ivory) and, perhaps most important, avert the awful and protracted suffering of the drought-driven mass die-off that must inevitably occur otherwise.

6. LOOKING TO THE FUTURE

Should sound animal husbandry principles and political backbone ever prevail to enable a resumption of professional cropping of elephant in the parks most threatened by overpopulation pressures, a concerted international scientific effort must be mounted to make best possible use of the veritable wealth of unique biological specimens that would arise as spin-offs from the cull. All aspects of whole body physiology and pathology should be covered by appropriate recording and sampling of significant tissues and body fluids and these samples should be analysed by recognized medical, veterinary and biological specialists in each particular field. Concerning reproduction especially, the many unanswered questions highlighted in this review and elsewhere (see Hodges 1998) with regard to follicular and luteal development in both pregnant and non-pregnant adult females, should be pursued vigorously, by giving special attention to the number, sizes and tissue-progestagen contents of follicles and/or any CL found in the ovaries of still non-pregnant lactational anoestrous females with 1–4 year old calves at foot, and, of course, during any observed periods of oestrus and in the very earliest stages of pregnancy. Similarly, in seeking a possible source of gonadotrophic stimulus for

both the maternal ovaries during the first half of gestation and the foetal gonads during the second half of gestation, placental tissue and maternal blood samples in very early gestation, together with foetal blood and foetal pituitary tissue samples during the second half of gestation, should be assayed for gonadotrophin, steroid and prolactin contents. And the endometrium of all adults, both pregnant and non-pregnant, should be examined grossly and histologically for signs of age-related degenerative, and/or pathological, changes which may yield clues to the incidence and significance of such changes observed ultrasonographically in captive female elephant (Montali *et al.* 1997; Hildebrandt *et al.* 2000a; Hermes *et al.* 2004). Finally, further attention should be paid to early post-partum uteri to attempt to unravel the cause and significance of the excessive amounts of intrauterine blood seen in such uteri to date (Allen *et al.* 2003).

Coincidentally with renewed culling and the associated collection and analyses of samples, it is clear that further veterinary and behavioural studies are required to ascertain the underlying causes of the very high levels of infertility currently recognized in captive Asian and African elephants (Sukumar 2003; Hermes *et al.* 2004). Very good between-zoo management collaborations, breeding policies and veterinary investigative techniques exist at the present time to undertake such studies, including the training of female elephants to accept serial blood sampling and transrectal ultrasonographic examinations of their reproductive tracts (Hildebrandt *et al.* 2000a), and the training of the adult males to accept vigorous transrectal massage of the accessory sex glands to induce ejaculation and thereby provide a representative sample of semen, both for analysis of sperm quantity and quality and for possible artificial insemination of oestrous females (Hildebrandt *et al.* 2000b). In the meantime, it seems reasonable to propose that more concerted management efforts to house, feed and generally manage captive elephants under conditions that more closely resemble the environmental, nutritional and hierarchical family traditions and behaviour patterns which pertain in the wild, and which have been catalogued and described so eloquently by Moss (1983, 1988) and Poole (1989) in their prolonged study of the Amboseli Park elephant population in southern Kenya would be most likely to bear the best fruit.

For example, the provision of very much larger enclosures containing appropriate browsing vegetation and walking trails to provide regular exercise, all in an attempt to limit weight gain, general body maturation and the onset of puberty in young female captives to more closely equate with those parameters in young wild equivalents (Laws 1969a; Stevenson 2005). And of equal or greater importance, to run young pre-pubertal female and male elephants together, as occurs in the wild, and to permit mating or insemination of the females as soon as possible after they pass through puberty and begin to display regular and hopefully ovulatory and fertile, oestrous cycles. Oestrus and associated ovulation may be presumed to be rare occurrences for wild females due to the constant succession of pregnancy followed by a 2–3 year post-partum lactation anoestrus followed again by renewed

pregnancy as soon as mating recurs. Perhaps it is the repeated 14–16 week ovarian cycles experienced by captive adult females (Kapustin *et al.* 1996), which are probably 'silent' in terms of expressed oestrous behaviour due to the absence of an adult musth male, that are most directly related to the endometrial cystic degeneration and leiomyomata formation observed ultrasonographically in relatively young captive females by Hildebrandt and his colleagues (Montali *et al.* 1997; Hermes *et al.* 2004). Even if they must be forced to live in zoos in unnatural climates and eating very artificial diets, let elephants be pregnant elephants together from a young age and normal reproductive patterns and fertility will very likely be resumed and maintained.

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