Chapter 1. General Introduction

1.1. Classification of elephants

Elephants belong to the order Proboscidea, family Elephantidae, of which there are thought to be two extant species, *Loxodonta africana*, the African elephant (Figure 1.1), of which the oldest fossils known are dated at 1.5 million years (Meredith 2001), and *Elephas maximus*, the Asian elephant (Meredith 2001). Recent debate has failed to draw a conclusion on whether *L. africana* is formed of two sub-species, *L.a. africana* and *L.a. cyclotis*, or whether *L.a.cyclotis* is a separate species (Blanc *et al*. 2007; Debruyne 2005; Roca *et al*. 2005). There is concern that premature allocation into more than one species may leave hybrids in an uncertain conservation status (Balfour *et al*. 2007) as it appears that where their natural boundaries cross, *L.a. africana* and *L cyclotis* may interbreed (Groves & Grubb 2000). Although the natural territory of *L. africana* and *E. maximus* do not overlap, a captive cross species mating has taken place and a live calf born which died 2 weeks later (Eltringham 1997). Pachyderm is another term of classification used to describe elephants, referring to an obsolete order of mammals grouping them with hippopotamuses and rhinoceroses because of their thick skin; the term is still used outside the boundaries of strict biological classification. Elephants' closest living relatives are thought to be *Sirenia* (Dugongs) and possibly *Procavia* (Rock hyrax).

Figure 1.1 The African elephant

a. A fully tusked group of African elephants, two females with two bull calves in Hwange National Park, Zimbabwe
b. A tusked matriarch with tuskless female offspring in Mana Pools NP, Zimbabwe

African elephants were historically found throughout Africa in habitats of deserts, forests, savannas, river valleys and marshes (Estes 1992). As far back as 5000 BC elephants were prized for their ivory in Egypt (Meredith 2001) but, due to climate change associated with the development of the Sahara desert, hunting, poaching and the encroachment of human settlement, elephants are now found only within restricted areas of the continent (Blanc *et al*. 2007). Their ecological importance as a keystone species is undisputed and elephants have a greater impact on their environment than any species other than man (Laws *et al*. 1970). Economically, they have been valued for their ivory which became known as "white gold", the relentless pursuit of which almost brought about the complete extirpation of the African elephant in the early 1900s (Marais & Hadaway 2006). In an attempt to restrict the trade in ivory the African elephant was placed on The Convention in International Trade in Endangered Species (CITES) Appendix III in 1976 and moved to Appendix II in 1977. Finally, in 1989, all African elephant populations were placed in CITES Appendix I, thereby banning international trade in elephants and their parts, including ivory. However, 1997 then saw the down-listing of Botswana, Namibia and Zimbabwe to Apendix II due to their healthy populations. Today there is a world-wide ban on trade in ivory but despite this poaching and an illegal trade in ivory does continue (Lemieux & Clarke 2009). African elephants are now valued by the tourist industry and in areas of overabundance the income generated from trophy hunting is a welcome boost for conservation projects (Lindsey *et al*. 2007). The discrepancy of "too few elephants here and too many there" has led to the systems for their protection in some African countries and to the necessity of reducing their numbers in others. Where there exists an overabundance of elephants alongside a growing human population an inevitable conflict of interests will arise, the solution to which will depend upon the value placed by man (indigenous and foreign) on these incredible creatures.

In Sections 1.2 to 1.4 follows an introduction to the broad field of knowledge on elephant reproduction, ranging from fetal development to reproductive senescence.

1.2. A brief overview of studies related to reproductive processes in the elephant

The reproductive organs of the female African and Indian elephants have been studied since 1734 (Chapman 1880; Forbes 1879; Patterson & Dun 1898; Perrault 1734). These

and other papers were summarized by Neuville (1937) and subsequently reviewed by Perry (1953). The latter's work included studies on ovaries collected from 81 females in Uganda between 1946 and 1950 and is widely accepted as the basis of modern knowledge of the reproductive system of the African elephant. It does, however, have a notable absence of information on folliculogenesis and the ovarian reserve, perhaps because little was known about follicle dynamics at the time. It wasn't until the studies by Zuckerman (1951) and Peters and Levy (1963) that the mechanism of follicle supply became fully appreciated (Gosden 1995).

During periods of culling in Uganda, Tanzania, Kenya and Zambia in the 1960s and 1970s several authors described the reproductive patterns of the elephant (Buss 1966; Cooper *et al*. 1964; Guy 1975; Hanks 1972; Hanks 1973; Hanks 1979; Hanks & Short 1972; Jainudeen *et al*. 1971; Kerr 1978; Laws 1967; Laws 1969; Laws *et al*. 1970; Ogle *et al*. 1973; Sherry 1975; Short 1966; Short 1969; Short & Buss 1965; Sikes 1971; Smith *et al*. 1969; Smith & Buss 1975; Watson & D'Souza 1975; Williamson 1976) but, again, without any description of early follicle development.

Following a growing trend toward a ban on culling elephants in the 1980s and early 1990s very few ovaries have been available for study in recent years and the few additional papers that have been published have been limited to gross descriptions of the ovaries and placentation (Allen 2006; Allen et al. 2005; Allen et al. 2003; Allen et al. 2002; Balke 1988; Gaeth et al. 1999; Glickman et al. 2005; Gunasena et al. 1998; Kidson et al. 1995; Wooding et al. 2005). In addition informative books reviewing historical, behavioural and other aspects of elephant natural history have been published (Moss *et al*. 2011; Spinage 1997; Sukumar 2003).

Endocrine and ultrasonographic techniques have replaced anatomical and histological studies since *post mortem* specimens have not been available for research. While these recent studies have not included small follicle counts they have, nonetheless, established the basic parameters of the elephant's reproductive cycle and are summarized in the review by Hildebrandt *et al*. (2011). The studies have been carried out predominantly on captive elephants in zoos across Europe and North America during investigations into the apparent decrease in fertility of captive females (Proctor *et al*. 2010).

Elephant contraception is an important field of study since, in southern Africa, there exists a need to reduce elephant numbers in isolated populations in order to prevent environmental damage and/or large-scale mortality of elephants due to overcrowding (Bertschinger *et al*. 2008). However, papers reporting studies on immunocontraception in elephants using porcine zona pellucida protein vaccine (pZP) mention the development and regression of follicles without reporting on their numbers.

In view of the longevity of reproductive life in the elephant the establishment of the early follicle population and its loss through life is of considerable interest. Although the view has been challenged in the last 10 years (see review by de Felici 2010) reproductive ageing in mammals is widely accepted to be irreversible due to oogonial stem cells disappearing after birth (Gosden & Lee 2010). However, the debate continues, especially since Zou *et al*. (2009) demonstrated the birth of live young from whole body irradiated mice following transplantation of mesenchymal stem cells to their ovaries in which all indigenous oocytes had been destroyed.

In the light of the above the aim of the current study, as reported in this thesis, was to investigate the establishment of the follicle reserve and the numbers of small follicles constituting this reserve throughout life in the African elephant.

1.3. The gross and microscopic anatomy and physiological functions of the elephant ovary and its follicles

1.3.1. Research pertaining to fetal, neonatal and prepubertal ovaries

In the absence of published data concerning the growth of the ovary in the elephant fetus it is necessary to assume that it develops in the same way as that of other mammals and, folowing sexual differentiation at around 105 days post conception (Glickman et al. 2005; Hildebrandt et al. 2007) is populated at an early stage by oocytes and their attendant granulosa cells. Allen *et al*. (2005) described the ovary of a 650 g elephant fetus (approximately 7 months of gestation) as displaying “a dense accumulation of oocytes in the cortical region, most of which were surrounded by a single layer of flattened pre-granulosa cells”. Cords of these primordial follicles extended towards the medulla with there being no sign of any follicle enlargement or interstitial cell development at this early stage. Interstitial cells, which possess the organelles associated with steroid hormone

synthesis, are present in the fetal gonads of many mammalian species, including humans (Konishi *et al*. 1986) and in some of these species hyperplasia and hypertrophy of interstitial cells results in an enormous increase in size of the fetal gonads which subsequently regress around birth. This enlargement of the fetal gonads occurs during the second half of gestation in the elephant (Allen *et al*. 2005), the horse (Deanesly 1977; Hay & Allen 1975) and grey seal (Hobson & Boyd 1984).

Enlargement of the fetal ovary in mid-gestation may be enhanced by antral formation in numerous growing follicles and their associated thecal layers which become broader and denser along with follicle growth. This occurs in the human (Lintern-Moore *et al*. 1974), the giraffe (Kellas 1958) and, particularly, the elephant (Allen *et al*. 2005) although as gestation continued past 18 months these authors noted a steady decline in the number of antral follicles.

According to Perry (1953), the combined weight of the ovaries reaches 80 g in the fetal elephant but falls to only 20 g in the neonate following regression of the interstitial tissue late in fetal life. Likewise, the ovaries of adult grey seals (*Halichoerus grypus)* weigh 7–27 g whereas those of seal fetuses reach 47 g in total weight (Hobson & Boyd 1984). And in the horse fetus at 250 days of gestation the combined weight of the fetal ovaries may be 50–100 g compared to only 10–20 g in the neonate (Hay & Allen 1975). This late gestation decline in fetal gonad weight in the mare parallels the decrease in concentrations of oestrogens in serum and urine (Cox 1975; Raeside & Liptrap 1975) and it was suggested by Allen *et al*. (2005) that the decrease in weight of the fetal gonad during late pregnancy in the elephant may similarly be associated with the slight decline in maternal serum progestagen concentrations during the same period, as reported by Meyer *et al*. (2004).

Around one year of age each ovary of an elephant calf weighs from 15 to 60 g (Perry 1953), interstitial tissue has become inconspicuous suggesting that the increased volume of the organ is due to growth of the other elements, particularly the connective tissue. The bulk of the cortex is reduced relative to the medulla and now constitutes a well-demarcated peripheral zone containing many fewer follicles than previously (Perry 1953). In the majority of prepubertal elephants the left ovary is heavier than the right and the same disparity is also present in the fetus (Hanks 1973).

1.3.2. Ovarian structures during different phases of the reproductive cycle of adult elephants

1.3.2.1 General morphology of the ovary

The ovary in the adult elephant is generally quoted as measuring, on average, 7 x 5 x 2 cm (Hildebrandt *et al*. 2000; Sikes 1971). The size and shape of the elephant ovary, however vary, resulting in a unique external morphology of each ovary. The surface of the ovary in cycling and pregnant elephants resembles that of the brain, possibly as a result of the restructuring occasioned by the multiple *corpora lutea* (CL) that develop during reproductive life (Hodges 1998; Lueders *et al*. 2011). In an earlier study (Stansfield 2006) it was noted that the ovarian surface in nulliparous animals was usually much smoother than in older multiparous elephants. Perry (1953) suggested that the deeply pitted surface of the elephant ovary may serve to extend the area of surface epithelium and although few in number and shallow in depth the undulations were nevertheless present in all the ovaries studied.

The morphology of the ovary is similar to that of most other mammals. A surface epithelium overlays the *tunica albuginea* beneath which is the cortical layer and, internally, the medulla and central hilus region leading to the mesovarial ligament which attaches the ovary to the uterus. The reproductive status of the animal contributes greatly to its ovarian morphology.

1.3.2.2 An overview of the endocrinology of the oestrous cycle of the African elephants

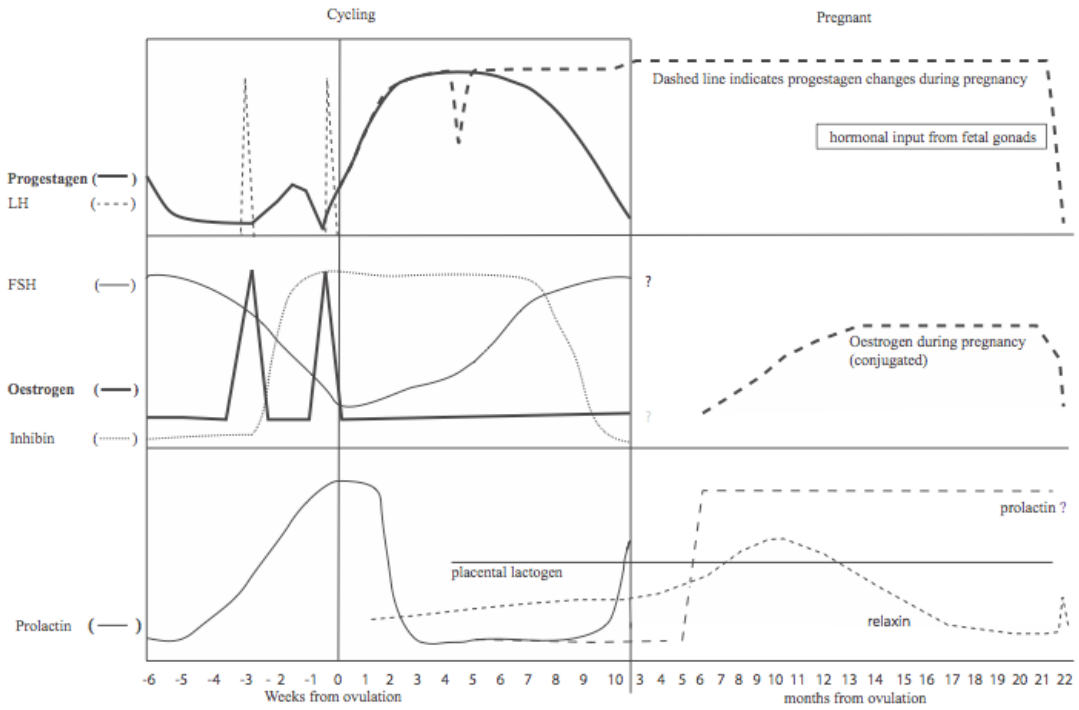
Before describing the structures on the ovaries of the elephant during various stages of the reproductive cycle, this section gives a brief overview of the endocrine changes during the oestrous cycle and pregnancy.

The oestrous cycle lasts 13–18 (16.3 ± 0.4) weeks and consists of a 6–12 (10.5 ± 0.3) week luteal phase and a 4–6 (5.1 ± 0.4) week follicular- or interluteal phase (Brown 2000; Brown *et al*. 2004a; Hildebrandt *et al*. 2011). A diagrammatic representation of the endocrinology of the oestrous cycle and pregnancy is shown in Figure 1.2.

Endocrine dynamics during the interluteal phase: The elevated serum FSH concentrations at the beginning of the follicular phase recruits follicles and stimulates two successive waves of follicular development each culminating simultaneously with one of two distinct, precisely timed, LH surges, the first of which occurs 12–21 days after progestagen concentrations have declined to baseline and the second 19–21 days later (Figure 1.2). This second LH surge induces ovulation about 24 hours later. The two LH surges are qualitatively and quantitatively similar but only the second induces ovulation (Brown *et al*. 1991; Brown *et al*. 1999; Lueders 2010).

Elevated oestrogen concentrations, which may not be measurable in the peripheral circulation during each follicular wave, trigger the LH releases but the follicles in the first wave have been considered functionally not competent to ovulate (Hodges 1998). More recent findings suggest ovulation may take place following the first LH surge but pregnancy does not ensue (Stansfield & Allen 2012). These follicles (with and without ovulation stigmata) are reported to luteinise in response to the first LH surge, thereby forming the luteal bodies destined to become the accessory CL of pregnancy (Lueders et al. 2012).

The concentration of inhibin (which is of follicular origin) in the blood is inversely related to that of FSH and positively correlated with that of progestagen (Brown *et al*. 1991) Inhibin concentrations remain basal until the latter part of the follicular phase, increasing after the first LH surge, peaking before the second LH surge and remaining elevated thereafter until the end of the luteal phase (Taya 2007). This suggests that, in the elephant, not only the granulosa cells of the follicles but also the CL secrete inhibin (Lueders *et al*. 2011; Taya 2007). In the cycling African elephant the concentration of prolactin in the serum is highest during the follicular phase (Brown *et al*. 2004b).



Data extracted from Brown, 2004; Hodges, 1998; Hildebrandt, 2011; Lueders, 2010; Yamamoto, 2011 and Meyer 2004

Figure 1.2 Hormonal changes in the peripheral blood of female elephants during the oestrous cycle and pregnancy (diagrammatic)

Endocrine changes in the luteal phase: Following the 2nd LH surge which stimulates the "fertile" ovulation, progestagen concentration in the blood increases as a reflection of the development and maturation of the CL (Figure 1.2). This is followed by a gradual rise in serum FSH concentration which peaks at the end of the luteal phase. Unlike most other mammals, FSH concentration is maximum at the beginning of the follicular phase and the level then declines to a minimum within 4 days after the ovulatory LH surge (Brown *et al*. 1999). This implies that the early stimulation by FSH is sufficient to provide prolonged stimulation for development of both the follicular waves that constite the follicular phase (Taya 2007). During the luteal phase there is no evidence of follicle development which only recurs once progestagen concentrations have returned to baseline (Hermes 2000; Lueders *et al*. 2010). The circulatory progestagens produced by both the ovulatory CL and the accessory CL of pregnancy are 5α-dihydroprogesterone and other 5α-reduced pregnanes (Heistermann 1997; Hodges *et al*. 1997). Serum oestrogen concentrations remain basal during the luteal phase (Kapustin *et al*. 1996).

Endocrine changes during pregnancy: The luteal phase of the oestrous cycle lasts approximately 10 weeks. During that period the embryo must signal its presence to the mother in order to block cyclic activity. Membranes without any associated embryo have been observed around 3–4 weeks post ovulation (Allen *et al*. 2003; Perry 1974). Serum progestagens increase after ovulation to a peak 3–4 weeks later. They then begin to decline before rising again steeply around 5–6 weeks, after which they remain elevated until mid-gestation. A second slow decline then occurs before they plateau until the end of pregnancy (Meyer *et al*. 2004). They are secreted by the primary and secondary *corpora lutea*, and very likely the secondary rise is stimulated by the luteotrophic action of elephant placental lactogen (elPL) after the first 5–6 weeks (Yamamoto *et al*. 2011), and they are likely supplemented by progestagens secreted by the interstitial cells in the enlarged fetal gonads during the second half of pregnancy; the placenta itself does not produce steroids (Allen 2006; Allen *et al*. 2003). Serum progestagen concentrations begin to fall 3–5 days before parturition (Meyer *et al*. 2004).

Following parturition, the elephant remains in lactation-related anoestrus for at least 22 months (Moss & Lee 2011).

1.3.2.3 Ovarian morphology during post-partum anoestrus

Just before parturition the large *corpora lutea* of pregnancy reduce slightly in size and begin to darken in colour. After birth they steadily reduce further over many months to eventually become small, irregularly-shaped blocks of dark brown or black tissue (*corpora nigra: CN*) distinguished histologically only by the presence of the pigment granules that are responsible for the colour (Perry 1953). Thus, the ovaries of non-pregnant lactating cows are distinguished by the absence of large active yellow or khaki-coloured, homogenous CL. During this post partum period Perry (1953) reported that a number of follicles grew to a diameter of about 5 mm but developed no further. Laws (1969), on the other hand, noted that the mean maximum follicle diameter in anoetrous lactating females was 4–6 mm, with occasional follicles measuring up to 10 mm. Using such ovarian morphology it then becomes possible to identify the reproductive status of a wild, post-partum female during *post mortem* examination with a good degree of accuracy.

1.3.2.4 Morphological changes in the ovary during the oestrous cycle

The possible mode of ovulation in the elephant has been discussed in many previous papers due to the presence of multiple large CL in the ovaries of pregnant females, more than one of which may have prominent stigmata (Hanks & Short 1972; Laws 1969; Perry 1953; Short 1966; Stansfield & Allen 2012). Hodges (1998) concluded that multiple CL, with and without ovulation stigmata, probably formed in successive oestrous cycles, with structural, but not functional, persistence into subsequent cycles as the most likely explanation of the visible evidence. Through the more recent ultrasound studies of Lueders *et al*.(2012) it has now been established that the multiple CL of pregnancy develop from luteinisation of medium sized non-ovulatory follicles after the first of the two LH surges that characterise the interluteal period of the elephant oestrous cycle (Brown *et al*. 1999; Kapustin *et al*. 1996; Lueders *et al*. 2011). In response to the second LH surge that occurs 20–22 days after the first, one larger Graafian follicle (16–21 mm in diameter) ovulates and produces a dominant CL. The several luteal structures that originate from the first LH surge persist in the ovaries (Lueders et al. 2012) and can be seen ultrasonographically to enlarge significantly some 35–50 days into gestation. This probably occurs in response to the commencing secretion of placental lactogen (elPL), a powerful luteotrophin, by the recently implanted trophoblast (Yamamoto *et al*. 2011) and results in a significant secondary rise in progestagen concentrations in maternal blood (Meyer *et al*. 2004).

With regard to the number of CL present in the ovaries of cycling elephants Laws (1969) reported that in 10 females, which had multiple CL in their ovaries but no visible conceptus in the uterus, CL numbers ranged from 3 to 22. It is noteworthy that an embryo aged 4 months weighs <5 grams so can be easily overlooked (Allen 2006). To complement what Laws found, Hanks and Short (1972) observed that non-pregnant adult elephants exhibited a high incidence of very small CL but a paucity of large ones.

Following considerable debate in previous years (Laws 1969; Short 1966; Short & Buss 1965) the maximum size of an ovulatory follicle, as monitored by repeated transrectal ultrasound examination, has been established as approximately 20 mm (Lueders *et al*. 2010). It is possible for the first oestrus after the post-partum lactational anoestrus period to be accompanied by a fertile mating and ovulation with the development of sufficient luteal tissue to maintain the ensuing pregnancy (Perry 1953).

1.3.2.5 Oestrus

Oestrus may last 24–48 hours (Short 1966), or up to 6 days (Skinner & Smithers 1990). A range of pre-copulatory behaviour patterns displayed by the male and female have been noted during more than 30 years of close study of elephant behaviour in Amboseli National Park in Kenya (Poole & Granli 2011).

1.3.2.6 Ovarian morphology following oestrus

Table 1.1 summarises the information obtained by Short (1966) from the ovaries of an elephant shot immediately after she had been observed being mated. This original observation concurs with present-day ultrasound investigations which report that elephants release a single oocyte per cycle while CL originating from 3 different timelines may be observed simultaneously in the elephant ovary (Lueders *et al*. 2010). The significance of the presence of stigmata, which Hodges (1998) observed on 30–40% of CL of apparently similar age is unknown. Lueders *et al*. (2010) suggest that stigmata may not reflect a point of ovulation, whereas Stansfield and Allen (2012) propose that ovulation may take place prior to luteinisation of follicles following the first LH peak.

Table 1.1
Ovarian structures in an elephant shot immediately after mating during oestrus; data from Short (1966)

Right ovary	Left ovary
Corpus luteum 1 (CL1)[a] 5 mm diameter and another (CL2) of 18 mm diameter, each with a clear ovulation stigma	One corpus luteum (24 mm diameter; CL3) and one fresh ovulation point 1–2 days old (9 mm diameter; CL4)
Eight brown regressing *corpora lutea*	Eight corpora nigra
One large Graafian follicle (12 mm)	Nine Graafian follicles (all< 5 mm diam.)
Ten smaller follicles (<5 mm)	

[a] CL 1, 2 and 3 were indistinguishable whereas CL 4 was well vascularised and was made up of two types of cells, one with rounded, reticulate nuclei and the other with densely staining nuclei.

1.3.2.7 Ovarian morphology during pregnancy

Follicles: Perry (1953) and Laws (1969) both recorded the presence of antral follicles in the ovaries of pregnant elephants. Perry (1953) observed that some follicles with antra up to 5 mm in diameter were present in all elephant cows during the first half of gestation ie. until the fetus weighs 8 kg, but no antral follicles occurred beyond this stage, suggesting that pregnancy-associated hormones may suppress follicular growth. Laws (1969) similarly noted that, unlike the follicles measuring 5–7.5 mm in diameter present during early pregnancy, no macroscopically visible follicles persisted by mid-gestation. Likewise, Smith and Buss (1975) reported the mean maximum diameter of Graafian follicles in pregnant elephant as 3.1 mm. A combination of the increased secretion of 5 α dihidroprogesterone and other 5α-reduced progestagens by the accessory CL (Heistermann 1997) and elPL (Yamamoto *et al*. 2011) may underlie this suppression of follicular growth during the second half of gestation.

Corpora lutea The ovaries of pregnant elephant are characterized by the presence of multiple *CL* that measure 5–35 mm in diameter and are frequently, but not always, confined to the ovary that is ipsilateral to the gravid uterine horn (Allen *et al*. 2002; Hodges 1998; Perry 1953; Short & Buss 1965). The luteal tissue is homogenous, yellow-to-khaki in colour and clearly active in terms of progestagen secretion (Hodges *et al*. 1997). The observed increase in weight of the ipsilateral ovary during pregnancy can be attributed almost entirely to the growth of these CL and the increased vascular supply involved in their development (Perry 1953); this author observed at least one CL in all but one of every pair of ovaries he examined throughout gestation. On the other hand Hanks and Short (1972) recorded 22 pregnant elephant that each had CL on only the ovary ipsilateral to the conceptus. Using transrectal ultrasonography, Lueders (2010) recorded that the maximum diameter of both the accessory *corpora lutea* formed by luteinisation of medium sized follicles after the first LH peak of the interluteal period and the ovulatory CL formed after the second LH peak was reached around 40 days after this second LH peak which had stimulated the fertile ovulation.

Several papers have been written about the CL of the elephant, addressing the type of ovulation they may result from and their size, weight and number (Allen 2006; Hanks &

Short 1972; Heistermann 1997; Hodges *et al*. 1997; Hodges *et al*. 1994; Laws 1969; Ogle *et al*. 1973; Short 1966; Short & Buss 1965; Smith *et al*. 1969; Smith & Buss 1975). More recent ultrasound studies (Lueders et al. 2010; Lueders et al. 2012) have gone some way to answering these questions but why so many large CL are produced, and whether they remain necessary and fully functional throughout gestation, still needs to be addressed. The CL can be numerous (Table 1.2) and large (Table 1.3 and Table 1.4) and many of them have a significantly greater diameter than the Graafian follicles from which they apparently originate (Allen 2006).

Table 1.2
Numbers of corpora lutea reported in the ovaries of pregnant elephants

Range of number of corpora lutea	Reference
≥ 50	Perry (1953)
2–42	Laws (1969)
2–26	Hanks and Short (1972)
3–8	Allen *et al*. (2002)

Table 1.3
Weights of luteal tissue reported in the ovaries of pregnant elephants

Weight	Reference
The ovaries of some pregnant elephants contain >200 g luteal tissue	Perry (1953)
Total weight of luteal tissue in both ovaries increased from 19 g initially to 38 g in mid-gestation and declined to 22 g at term	Laws (1969)
All elephants in the first half of gestation have > 17.3 g luteal tissue	Hanks and Short (1972)
Individual CL weighed up to 30 g	Stansfield (2006)

Table 1.4
Range in luteal size reported in pregnant and cycling elephants

Status	CL diameter	Reference
Pregnant	3–6 cm	Perry (1953)
Cycling	2.4–3.3 cm	Hanks (1972)
Cycling	2.3–3.8 cm	Hermes (2000)
Pregnant	3–6 cm	Allen (2006)
Cycling	up to 4.1 cm	Lueders (2010)

Perry (1953) suggested it was possible to distinguish histologically as many as three generations of CL in a single pair of ovaries, the newest having developed before the oldest has regressed sufficiently to have lost its definite form. Lueders *et al*. (2010) now propose that these three generations may represent the anovulatory and ovulatory CL from one oestrous cycle together with the regressing CL from the previous cycle. Regressing CL are commonly termed *corpora rubra* or *corpora nigra (CN)*, to reflect the darkening colour of the degenerating luteal tissue (Smith & Buss 1975).

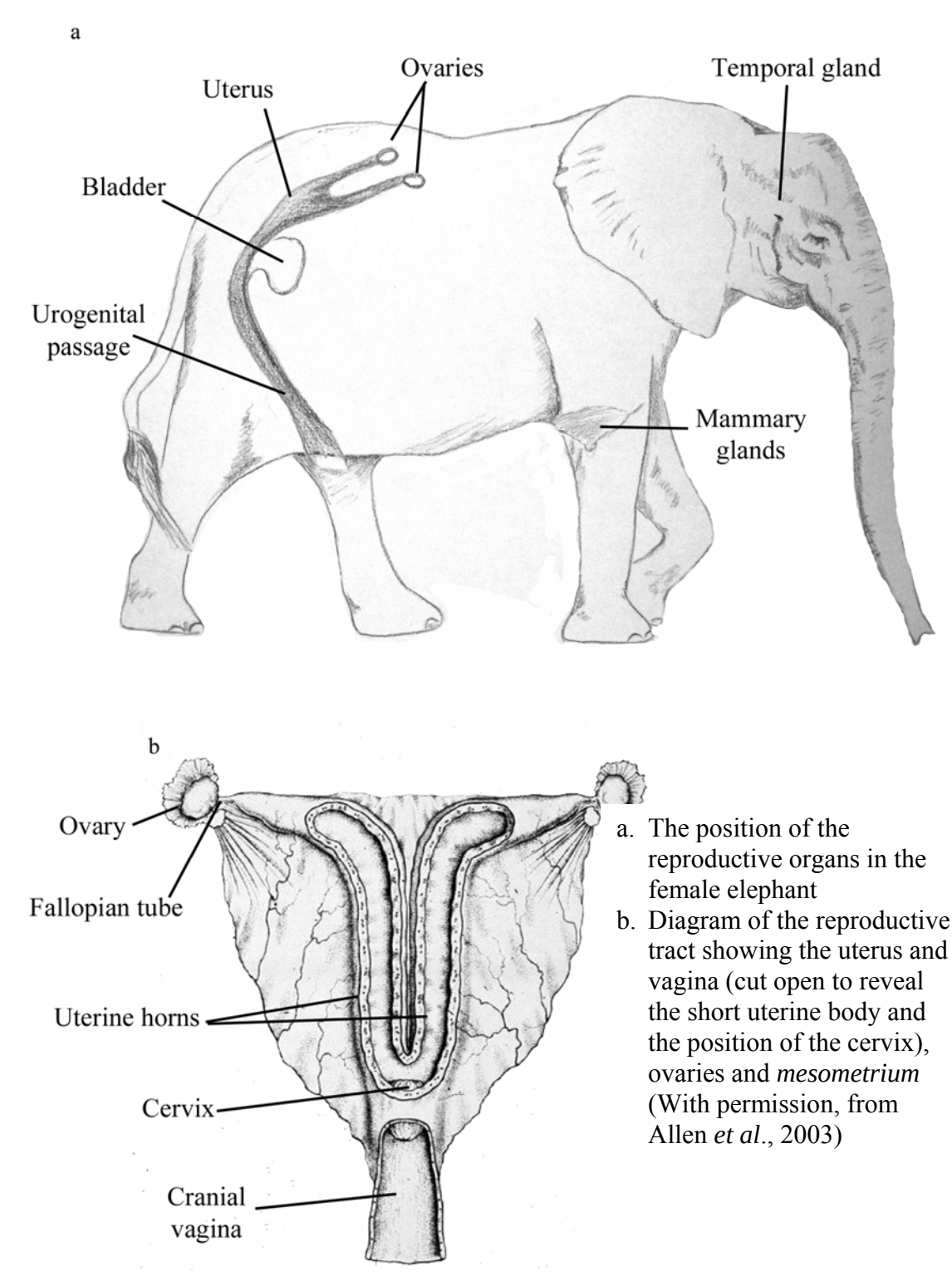
Not surprisingly, the development of the multiple large CL of pregnancy causes extensive remodelling of the ovarian cortex. In a previous study (Stansfield 2006) these large CL were dissected from the ovaries of pregnant elephants prior to serially sectioning the remaining ovarian tissue for histological examination. However, during subsequent follicle counts the expected large numbers of primordial follicles and abundant ovarian reserve could not be identified, which raised the suspicion that the development of the CL had caused sufficient distortion of the ovarian cortex to mask the small follicle populations. The physical impact of the size and number of CL on displacement of the ovarian cortex has not yet been described.

1.4. Further anatomy and physiology of the elephant

1.4.1. Anatomy of the female reproductive system

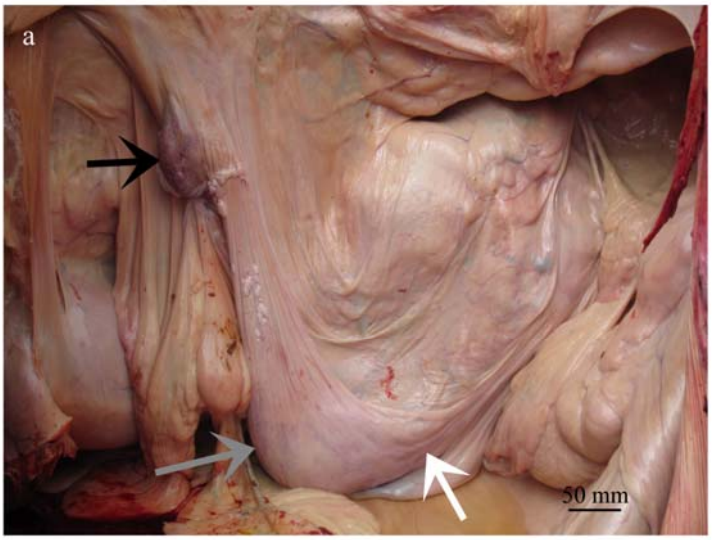
The female reproductive system of the elephant Figure 1.3 and Figure 1.4) comprises two ovaries situated close to the kidneys, comparatively short Fallopian tubes (10–15 cm in

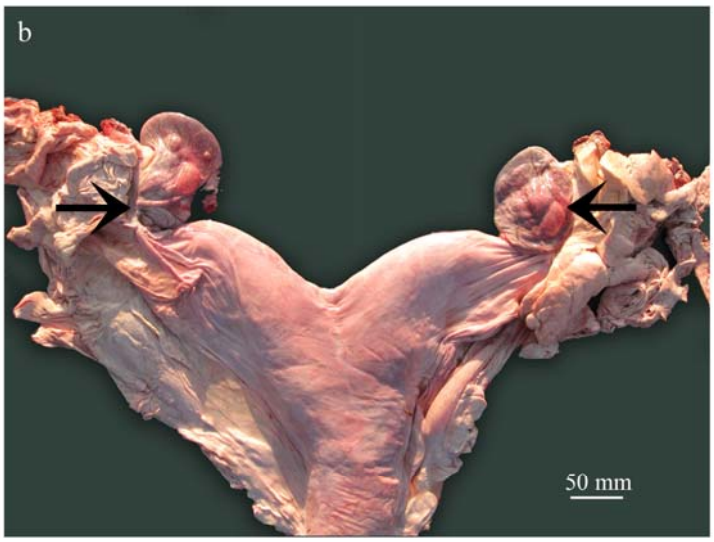
their semi-convoluted state *in situ*), a uterus consisting of two long cornua and a short body leading to a relatively short fibrous cervix. Caudal to the cervix lies the short cranial vagina (30 cm) separated by an almost complete hymen membrane from the long (1.0–1.4 m) urogenital passage, which opens externally at the vulva situated ventrally between the back legs and not in the perineum as in most other mammals (Sikes 1971). The ovary itself is almost completely enveloped by an expansion of the fimbrial funnel of the Fallopian tube. The latter forms a bursa, which is generally referred to as the ovarian sac (Perry 1953), the outer wall of which is covered with peritoneal epithelium while the inner surface is a very luscious mucosa. The ovarian bursa is connected laterally to the ovarian ligament and is open medially. The ovary is anchored to the uterine horn by the ovarian ligament and, cranially, to the body wall by the very short and fibrous suspensory ligament (Perry 1964). Both ligaments are well developed and strong and are contiguous with the hilum of the ovary. The length of the uterine (Fallopian) tube is around 25–30 cm when fully extended which is similar to that of many ungulates (Perry 1964). The lumenae of the uterine horns remain separate for some distance caudal to their point of external fusion and then open into the short common body of the uterus. The embryo probably implants ipsilateral to the side of ovulation, and usually in that part of the uterine horn that is fused externally with the contralateral horn (Sikes 1971).



a. The position of the reproductive organs in the female elephant
b. Diagram of the reproductive tract showing the uterus and vagina (cut open to reveal the short uterine body and the position of the cervix), ovaries and *mesometrium* (With permission, from Allen *et al*., 2003)

Figure 1.3 A schematic representation of the reproductive organs of the female African elephant





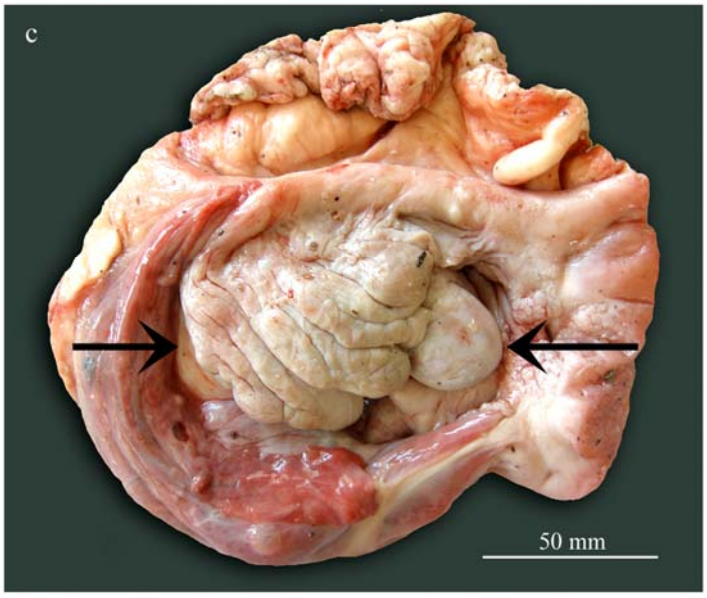


Figure 1.4 The uterus and ovaries of the African elephant

a. Left lateral view showing the ovary (black arrow), uterine horn (grey arrow) and uterine body (white arrow) *in situ*
b. Ventral view of the uterus and ovaries (black arrows) inside the ovarian bursae
c. The ovary (between arrows) attached to the mesovarium (top), with the ovarian sac (bursa) removed medially

1.4.2. Puberty

Laws (1969) classified female elephants on the basis of macroscopic examination of sliced ovaries into; i) immature — ovaries containing neither large follicles nor *CL* or *CN*; ii) pubertal — having no *CL* or *CN* but at least one follicle larger than 5 mm in diameter; iii) mature — having at least one CL or *CN in* the ovary. The age of puberty recorded in wild African elephants is shown in Table 1.5.

In captivity pregnancy has occurred at ages as young as 3.5 years in the Asian elephant and 7 years in the African elephant (Hildebrandt *et al*. 2011). Pregnancy usually does not occur until 10–12 years of age in the wild (Laws 1969).

Table 1.5

Age of puberty in African elephants

Age (years)	Country	Reference
Mean age at first breeding		
8–12	Uganda	Perry (1953)
11–20	East Africa[a]	Laws (1969)
12	Kenya	Moss (2001)
Mean age at first ovulation		
10–11	East Africa[a]	Laws (1969)
14	Zambia	Hanks (1972)
12–13	Zimbabwe	Sherry (1975)
11 (range 9–15)	Zimbabwe	Williamson (1976)

[a] The mean age of sexual maturity increases with increasing density-dependent physiological, nutritional and social stresses.

1.4.3. Fertility

Following 2 or 3 matings with the dominant bull while visibly in oestrus, the wild African elephant regularly conceives a singleton conceptus, and very rarely gestates twins (Allen 2006; Seth-Smith & Parker 1967). Laws (1969) put the incidence of twinning at 1.35% which is similar to other large herbivores.

Laws (1969) concluded that density-dependent natural regulatory mechanisms operate in elephant populations to induce changes in reproductive rate, predominantly by delaying the age of first calving but also by extending calving intervals (Table 1.6). Hanks (1972) noted peak fertility in cows aged 18–19 years with a reduction from about 40 years of age onwards. He did, however, observe macroscopically visible follicles in all but one of the 5 oldest female elephants in his study. From the published data summarized in Table 1.7 and Table 1.8 it appears that elephant fertility begins to decrease after 40 years of age, with a sharp decline beyond the age of 50 years. Nevertheless, some individuals do continue to breed into their 7th decade. Together these findings indicate that, despite their high level of fertility in younger life, fecundity does decline steadily beyond 50 years of age to leave females with a relatively short post-reproductive lifespan before death occurs as a result of starvation induced by disintegration of the final 4 molar teeth.

The high fertility of the large mating bulls and the mature cows result in high levels of conception and successful pregnancy (Moss 1996). Due to the infrequency of pregnancy loss, high postnatal survival rate and longevity, the African elephant, if not killed by man, is able to increase its population size by a remarkable 8% per annum (Whyte 2001).

Table 1.6
Published data on intercalving intervals in African elephants

Area	Intercalving interval		Reference
	Mean	Range	
East Africa	4	2.8–13	Laws (1969)
Uganda	3.8	3–4	Perry (1953)
Gonarezhou NP, Zimbabwe	3.7	3.3–4.6	Sherry (1975)
Luangwa Valley, Zambia		3.5–4	Hanks (1972)
Tanzania		4–5	Laws (1969)
Tsavo National Park NP, Kenya		6–7	Laws (1969)
Murchison Falls North NP, Uganda		6–7	Laws (1969)
Murchison Falls South NP, Uganda		8–9	Laws (1969)
Hwange NP, Zimbabwe	4		Williamson (1976)
Amboseli NP, Kenya	4.5		Moss (2001)

Table 1.7
Studies reporting on fertility in old African elephants

Published observations	Animals studied	Author
Fertility decreases after the age of 40 years	499	Hanks (1972)
In no case could it be shown conclusively that a female had ceased to breed.	81	Perry (1953)
High fertility in females aged 12–49 years with a sharp decline after 50 years when 32% were either not pregnant or lactating.	1737	Sherry (1975)
No significant peak in fertility between 13 and 49 years of age when approximately 50% of females were pregnant. The rate fell to 17% between 50 and 60 years of age, nevertheless, of the 17 elephants older than 50 years, 15 were lactating.	614	Williamson (1976)
All females aged 56–60 years were reproductively inactive, similar to menopause in women.		Laws *et al*. (1970)
Most females older than 50 years continued to reproduce but their intercalving intervals extended to 4.75 years from a population average of 4.5 years.		Moss (2001)

Table 1.8
Reproductive status of elephants older than 50 years culled in Kruger National Park, South Africa during 1975–1995. Data from Freeman *et al*. (2008).

	Number (and percentage) of elephant				
Age (years)[a]	Studied	Lactating	Pregnant	CL or CN in their ovaries	No ovarian activity (%)
50	8	7 (88)	3 (38)	7 (88)	0
51	12	10 (83)	8 (67)	11 (92)	0
52	12	7 (58)	4 (33)	8 (67)	1 (8)
53	6	6 (100)	2 (33)	6 (100)	0
54	4	2 (50)	1 (25)	1 (25)	0
55	10	7 (70)	5 (50)	4 (40)	1 (10)
56	4	1 (25)	0	3 (50)	1 (25)
57	0				
58	3	2 (68)	1 (33)	0	1 (3)
59	0				
60	5	0	0	2 (40)	3 (60)
Total	64	42 (66)	24 (38)	11 (17)	7 (11)

[a] Aged according to Laws (1966).

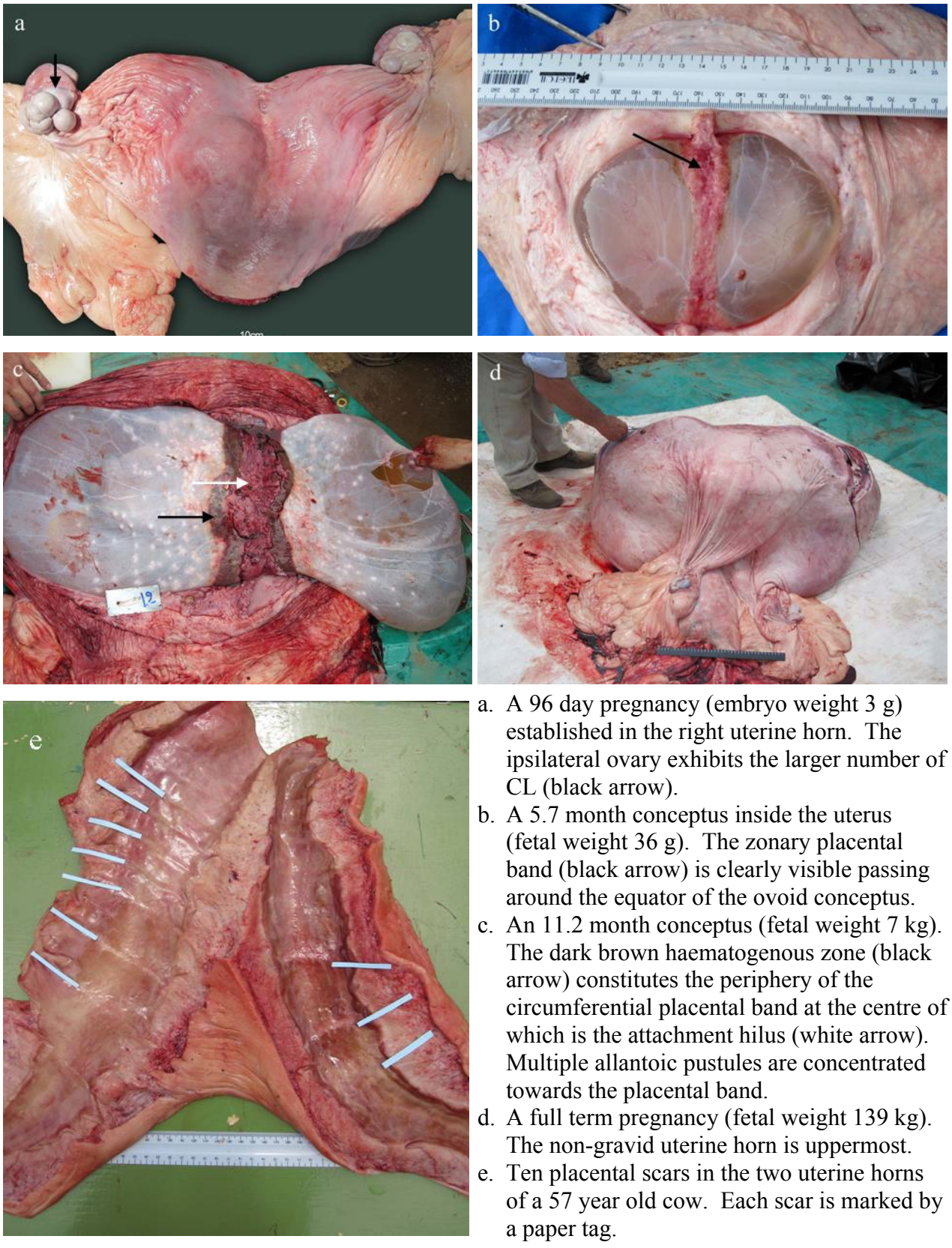
1.4.4. Fetal size

Craig (1984) established a formula for ageing elephant fetuses based on their weight; $t = 106w^{1/3} + 138$, where t refers to the age in days since conception and w the mass in kilograms. This has recently been superceded in the first third of gestation by a formula based on transrectal ultrasonographic measurements of fetal size; age = 28.434 + 54.20 x $(\text{mass})^{1/6}$ (Drews *et al*. 2008; Hildebrandt *et al*. 2007). Maximum fetal weight is approximately 120 kg at 22 months of gestation and a fetus below 8 kg weight is probably in the first half of gestation (Perry 1953).

1.4.5. Placentation

Sometime during the first 10 days after fertilization the embryo enters the uterus and becomes lodged in one of the 4 lateral clefts which cause the endometrial lumen to be star-shaped in cross section (Amoroso & Perry 1964; Perry 1974). During the first 40–60 days of gestation, pregnancy can only be "guessed at" by the presence of multiple large CL on the ovaries and a barely discernible conceptus bulge of only 2–3 cm diameter external protrusion situated near the lateral flexion of the ipsilateral uterine horn (Allen 2006). Between 2 and 3 months of gestation, when the fetus weighs 1–5 g, it is possible to discern the pale ribbon-like thickening which constitutes the developing placental band over the equatorial region of the ovoid conceptus (Allen 2006). A recent paper demonstrated that the African elephant trophoblast secretes a placental lactogen (elPL) from an early stage of gestation (Yamamoto *et al*. 2011). The authors proposed that elPl may stimulate the enlargement and secretory function of the accessory CL of elephant pregnancy and also provide the mitogenic stimulus for placental differentiation and development. In the final stages of gestation, the zonary placental band is 30 cm wide and 13–15 cm deep in its centre (Figure 1.5). It is attached to the endometrium via a narrow (2–3 cm wide) fibrous pedicle or hilus of maternal tissue through which passes endometrial stroma and blood vessels which, together, constitute the maternal component of the placental band (Allen 2006).

In uteri of elephants that calved recently a pronounced scar can be observed in the endometrium passing around the circumference of the previously gravid horn indicating the site of attachment of the placental hilus of the previous conceptus. These placental scars persist throughout life and counting them *post mortem* enables an accurate assessment of the parity status of the individual Figure 1.5e).



a. A 96 day pregnancy (embryo weight 3 g) established in the right uterine horn. The ipsilateral ovary exhibits the larger number of CL (black arrow).
b. A 5.7 month conceptus inside the uterus (fetal weight 36 g). The zonary placental band (black arrow) is clearly visible passing around the equator of the ovoid conceptus.
c. An 11.2 month conceptus (fetal weight 7 kg). The dark brown haematogenous zone (black arrow) constitutes the periphery of the circumferential placental band at the centre of which is the attachment hilus (white arrow). Multiple allantoic pustules are concentrated towards the placental band.
d. A full term pregnancy (fetal weight 139 kg). The non-gravid uterine horn is uppermost.
e. Ten placental scars in the two uterine horns of a 57 year old cow. Each scar is marked by a paper tag.

Figure 1.5 The placenta of the African elephant

1.4.6. Lactation

Perry (1953) observed that lactation overlaps the subsequent pregnancy in the great majority of adult females so that lactation becomes a continuous process throughout reproductive life. Suckling two siblings of different ages simultaneously has been recorded (Douglas-Hamilton 1972). By inference, therefore, the supposed influence of lactation on the presence or absence of Graafian follicles in the ovaries (Smith & Buss 1975) is not absolute.

1.4.7. Ovarian ageing in elephants

Te Velde and Pearson (2002) observed that, in the human female, an almost unique aspect of meiosis is the requirement for individual oocytes to remain meiotically competent for up to 40 years. They further noted that "the only other mammals with a remotely comparable length of female fertility are whales and elephants for both of which there is no information available as to the cause of the eventual cessation of fertility". It appears that elephant oocytes, formed during fetal life as in women, experience meiotic arrest at the dictyate stage so that genetic information is retained within the germinal vesicle until ovulation (Kidson *et al*. 1995). The effects of ageing on elephant oocytes and their chromosomes may be equivalent to that in humans at comparable ages, yet wild African elephants commonly continue to reproduce into their 6th decade and some into their 7th (Hanks 1972; Laws *et al*. 1970; Moss & Lee 2011; Perry 1953; Sherry 1975; Williamson 1976). Human fertility drops off slowly from a peak at around 25 years of age and may decline more sharply from around 37 years, failing completely at menopause around 50 years due to a reduction in the number and quality of oocytes in the ovaries (Faddy 2000; Gosden 1987). Counting the numbers of oocytes that constitute the follicle reserve in the elephants ovary at various times in life would therefore offer an interesting comparison to the existing human studies.

1.4.8. Elephant age classification

The ageing of elephants according to molar progression is described fully Chapter 2. Sykes (1971) provided a useful classification of elephants into various age groups based on ovarian morphology, as shown in Table 1.9. Laws (1969) described the attainment of sexual maturity and these two approaches were used initially in the present study.

Table 1.9
Age classifications of African elephants based on Sykes (1971) and Laws (1969)

Age group	Age (years)	Additional description of ovarian development
Calves	0–5	
Sexually immature juveniles	5–10	Large follicles may be present but no CL or CN.
Pubertal	8–15	No CL or CN but at least one follicle ≥ 5mm in diameter
Sexually mature sub-adults	10–15	At least one CL or a CN is present in the ovaries
Prime adults	15–35	
Senior adults	35–50	
Old	≥50	

1.5. Follicle development in mammals

1.5.1. Origin of the ovarian reserve

1.5.1.1 Primoridal germ cells

The pluripotent cells destined to, among others, give rise to the primordial germ cells, are first recognised in the proximal epiblast, from where they migrate to an extra-embryonic region posterior to the primitive streak, in the extra-embryonic mesoderm, near the site of origin of the exocoelomic cavity. There, some of these cells, now situated at the base of the developing allantois, become committed as primordial germ cells (PGCs). Once the endoderm starts invaginating to form the hindgut these committed PGCs are carried along with the endoderm along the length of the gut. Due to the nature of the invagination of the endoderm the PGCs are initially situated in the ventral aspect of the hind gut, from where they migrate dorsally, around the gut and further to the coelomic angle, and from there laterally to the genital ridge (McLaren 2003). The embryonic gonad develops from the germinal ridge of intermediate mesodermal origin and lies medial to the mesonephros (Langman 1981). Following the arrival of the germ cells into the indifferent gonad, primitive sex cords of the coelomic epithelium grow into the underlying mesenchyme,

invading both the presumptive cortical and medullary regions. Differentiation into ovary or testis depends on the embryonic genotype of the gonadal tissue (Adams & McLaren 2002). Only PGCs that reach the gonadal ridges survive. Those that remain outside in neighbouring tissues and organs undergo apoptosis at various stages of development (McLaren 2001; Zamboni & Upadhyay 1983), perhaps as a defence against their ability to become neoplastic under the influence of certain growth factors (de Felici *et al*. 2005). The chronology of events during gonadal differentiation in different species is shown in Table 1.10.

Table 1.10
Chronology of events during the differentiation of the mammalian gonad in various species (Days post conception)

Species	Cattle	Sheep	Pig	Horse	Dog	Cat	Mouse
Germ cells in the gonadal ridge	<35[a]	30–32	20	21	28		
Gonadal sex differentiation	26	40	52	60	28	28	9
Development of oogonia	57	43	28				
Onset of meiosis	70–82	55–60	40–70	73–90	At birth	40–50	10
First primordial follicles	90	66	64		3 pp	11 days after birth	
Mitosis of oogonia ends	160	82	100		15–17 days after birth	8 days after birth	
	228[b]						
First primary follicles	140	95					
First secondary follicles	210	103			60 days after birth		
First tertiary follicles	230	150		At birth	6 months after birth		
Gestation	280	150	115	336	62	63	20

The table contains combined data from (Adams *et al*. 2008; Latshaw 1987; Russe & Sinowatz 1991; van den Hurk & Zhao 2005).

1.5.1.2 Mitotic division

Primordial germ cells are so named until they reach the gonadal ridge (Byskov & Nielsen 2010). Once there and while they are dividing mitotically in the gonadal ridge, they are called oogonia, or germ cells if the embryo is of indeterminate gender. They become termed oocytes once they enter the process of meiosis. On arrival at the gonad, the final few rounds of mitosis result in the formation of groups of oogonia (2n2c), often called germline cysts or nests and connected by cytoplasmic processes (Pepling 2006). This mitotic proliferation has been observed up to 7.5 months of gestation in domestic cows, the number of oocytes per fetus reaching approximately 2 million (van den Hurk & Zhao 2005). In mice, oogonia proceed through approximately 4 mitotic cycles before entering meiosis (Gosden 1995).

Mitotic multiplication is key to the establishment of the future oocyte population as it is the balance between mitotic production and the apoptotic elimination of oogonia which determines the number of oocytes available for reproductive life in the mammalian female (Aitken *et al*. 2011). The signal for oogonia to stop mitotic division and start meiotic differentiation is unclear but it is thought to be associated with the presence of retinoic acid originating from the mesonephros (Bowles & Koopman 2007; Childs *et al*. 2011).

1.5.1.3 Meiotic division

In cattle oogonia start entering meiosis by 70 days after conception and, following a period of DNA synthesis around 75–80 days (Adams *et al*. 2008), the germ cells become arrested in the diplotene stage of the prophase of the first meiotic division at approximately 140 to170 days (Adams *et al*. 2008; Baker & Hunter 1978) when they are termed primary oocytes (2n4c). In oocytes that do not become atretic, meiosis only resumes following the onset of puberty and immediately prior to ovulation, giving rise to a secondary oocyte with a chromosome and chromatin configuration of 1n2c (Fair 2003). It has been suggested that in animals with longer gestation periods (ie. >40 days) there is a delay in the onset of meiosis and the associated gonadal differentiation which may enable blastemal (pre-granulosa) cells to proliferate, therefore allowing more germ cells to be incorporated into future follicles (Latshaw 1987). The duration of the period during which oogonia enter meiosis is also suggested to be extended with longer gestation

periods; 52–82 days in the ewe, 40–100 days in the sow, 70–170 days in the cow, 70–140 days in the female macaque and 82–160 days in woman. In the hamster, rabbit, ferret and dog the entry into meiosis is post natal (Deanesly 1977). The duration of a meiotic prophase from onset to the time of arrest is estimated to be around one week in the mouse, rat and hamster and 12–15 days in the rabbit, dog, pig monkey and human (Deanesly 1977). Such data is not presently available for the elephant.

1.5.1.4 Formation of the ovarian reserve

Coelomic epithelial cells of mesonephric origin give rise to both the primitive sex cords and the ovarian surface epithelium (Sawyer *et al*. 2002). The primitive sex cords are termed cortical cords peripherally and medullary cords internally and from 7 weeks of gestation in the human, the cortical cords develop and the medullary cords regress (Langman 1981). The genital ducts are also of mesonephric origin and remnants of the mesonephric duct in the female form the rudimentary rete ovarii (Sweeney 1998). Contact between the primary oocytes resident in the gonadal ridge and the pre-granulosa cells derived from either the ovarian surface epithelial cells (Sawyer *et al*. 2002) or the epithelial cells of the rete ovarii (Zamboni 1982), stimulate the pre-granulosa cells to form a basal lamina along their contact with the mesenchymal cells of the immature ovary. In this way the ovigerous cords are formed, consisting of oocytes and their associated pre-granulosa cells and reaching from the outer presumptive cortex to the inner presumptive medulla. The pre-granulosa cells develop cytoplasmic processes which attach to the plasma membrane of the oocyte, and the laying down of a basal *lamina,* to surround these now-termed granulosa cells (Pepling 2012) with the oocyte they enclose, marks the establishment of a primordial follicle, with its oocyte arrested in the germinal vesicle stage (Pepling 2006). This sequence of changes has been confirmed in women (Faddy & Gosden 1995), sheep (Lundy *et al*. 1999), rats (Oktay 1995) (Meredith *et al*. 2000), cows (Wandji *et al*. 1996) (Van Wezel & Rodgers 1996), baboon (Wandji *et al*. 1997), the yak (Cui & Yu 1999), and the monkey (Nichols *et al*. 2005). Oocytes that do not become associated with pre-granulosa cells degenerate (Picton 2001). This occurs in around 90% of the germ cells in the bovine fetal ovary thereby suggesting that the ovarian environment controls the progress through meiosis (Oktem & Urman 2010).

During oogenesis in all species, peak numbers of germ cells are observed around the time of the transition from mitosis to meiosis (Reynaud & Driancourt 2000). At this stage the

mean germ cell store of a cow is 2.1 x 10^6 (Erickson 1966). From their peak at embryonic day 13 (E13) in the mouse, E80 in cattle and E110 in woman, the number of observed germ cells decreases sharply, with two main periods of high germ cell loss; i) the pachytene (recombination) stage of meiosis in the oocyte and ii) the time of formation of primordial follicles (Aitken *et al*. 2011; Reynaud & Driancourt 2000). As a consequence of these losses the number of germ cells enclosed in primordial follicles at birth in the cow is less than 5% of the peak number. Apoptotic death at this time has been associated with anti-apoptotic factors (the BCL2 family of proteins, fibroblast-like growth factor [FGF], leucocyte inhibitory factor [LIF] and KIT/KIT-ligand) and pro-apoptotic factors (Bax protein, transforming growth factor-beta [TGF-ß] and FAS ligand), but the full mechanism has still to be defined (Aitken *et al*. 2011). The reason for this great deletion of the majority of newly formed oocytes so early in life needs to be elucidated. Speculation as to the means of depletion has included, i) death due to defect — involving removal of poor quality oocytes (Pepling & Spradling 2001); ii) death by self sacrifice of individual oocytes to allow pre-granulosa cells to penetrate between remaining oocytes (Pepling & Spradling 2001); iii) provision of nurse cells whereby the pre-granulosa cells of dying oocytes are adopted by near neighbours and, iv) death by neglect where oocytes may not receive the required local stimuli or somatic cell support (Tilly 2001). The highest germ cell survival rate in domestic mammals is found in the pig where approximately half the maximum number of germ cells survive until birth (McGeady *et al*. 2006).

The histological observation of the ovaries of the elephant embryo or fetus during its 22 month gestation may reveal an expanded period of the morphological and physiological development of these organs. Also of special interest in the elephant ovary is the development of antral follicles up to a diameter of 5 mm and large numbers of interstitial cells which, together, result in significant enlargement of the elephant fetal ovary during the second half of pregnancy (Allen *et al*. 2005).

Table 1.11 gives the number of follicles present in the ovarian reserve of various species throughout life.

Table 1.11
Numbers of primordial follicles in the ovary of mammals at varying ages (or number of oocytes during gestation)

Age or stage of gestation	Mouse[a]	Rat[b]	Domestic dog[c]	Domestic cat[d]	Sheep[e]	Horse[f]	Cow[g]	Human[h]
Peak number	250 x 10^3[i]	50–75 x 10^3			9 x 10^6 (75 d)		2.7 x 10^6 (110 d)	7 x 10^6 (5 mo)
Early gestation							16 x 10^3 (50 d)	
Mid gestation					200 x 10^3		107 x 10^3 (170 d)	
Late gestation					82 x 10^3		68 x 10^3 (240 d)	
Birth	7 924	10–15 x 10^3 [j]	700 x 10^3		82 x 10^3[j]		135 x 10^3[j]	1.5 x 10^6
Early life	1 987 (7 d)	924 (4–5 mo)	350 x 10^3		27 018(2 y)[k]			
Puberty		5 180[d]	150 380[d]	74 520	30–50 x 10^3	35 950 (2–4 y)	120 x 10^3 [o]	300 x 10^3
Old age	254 (200 d)		500 (10 y)		10 915[k]		3 x 10^3 [l]	
Max. reproductive life[m]			9	14	16–18	25	25	42
Max. life expectancy[m]			15	20	19	25	30	77+

[a] (Kerr *et al*. 2006); [b] (Meredith *et al*. 2000); [c] (McGeady *et al*. 2006); [d] (Gosden & Telfer 1987); [e] (Gondos 1978); [f] (Driancourt *et al*. 1982); [g] (McGeady *et al*. 2006); [h] (Faddy *et al*. 1992); [i] (Tam & Snow 1981); [j] (van den Hurk & Zhao 2005); [k] (Driancourt *et al*. 1985); [l] (Spicer & Echternkamp 1986); [m] (Cohen 2004)

1.5.1.5 Comparative and gross microscopic description of bovine ovaries

The ovary of the domestic cow and that of the elephant are broadly similar in morphology. Bovine ovaries from mature cows are approximately 3 x 2 x 1.5 cm in sizeand have 5 identifiable cortical zones (Van Wezel & Rodgers 1996) against which the cortical region of the elephant ovary may be compared:

Zone 1; The surface epithelium sitting on a basement membrane. This is a single layer of cells, either cuboidal or elongated, covering the surface of the ovary. Because this superficial epithelium does not revert to mesothelium the ovary is not covered with peritoneum in the adult and ovulation may occur at any point on the ovarian surface (Latshaw 1987). *Post-mortem* studies have revealed that the elephant has a similar surface epithelium to that of the cow (Perry 1953).

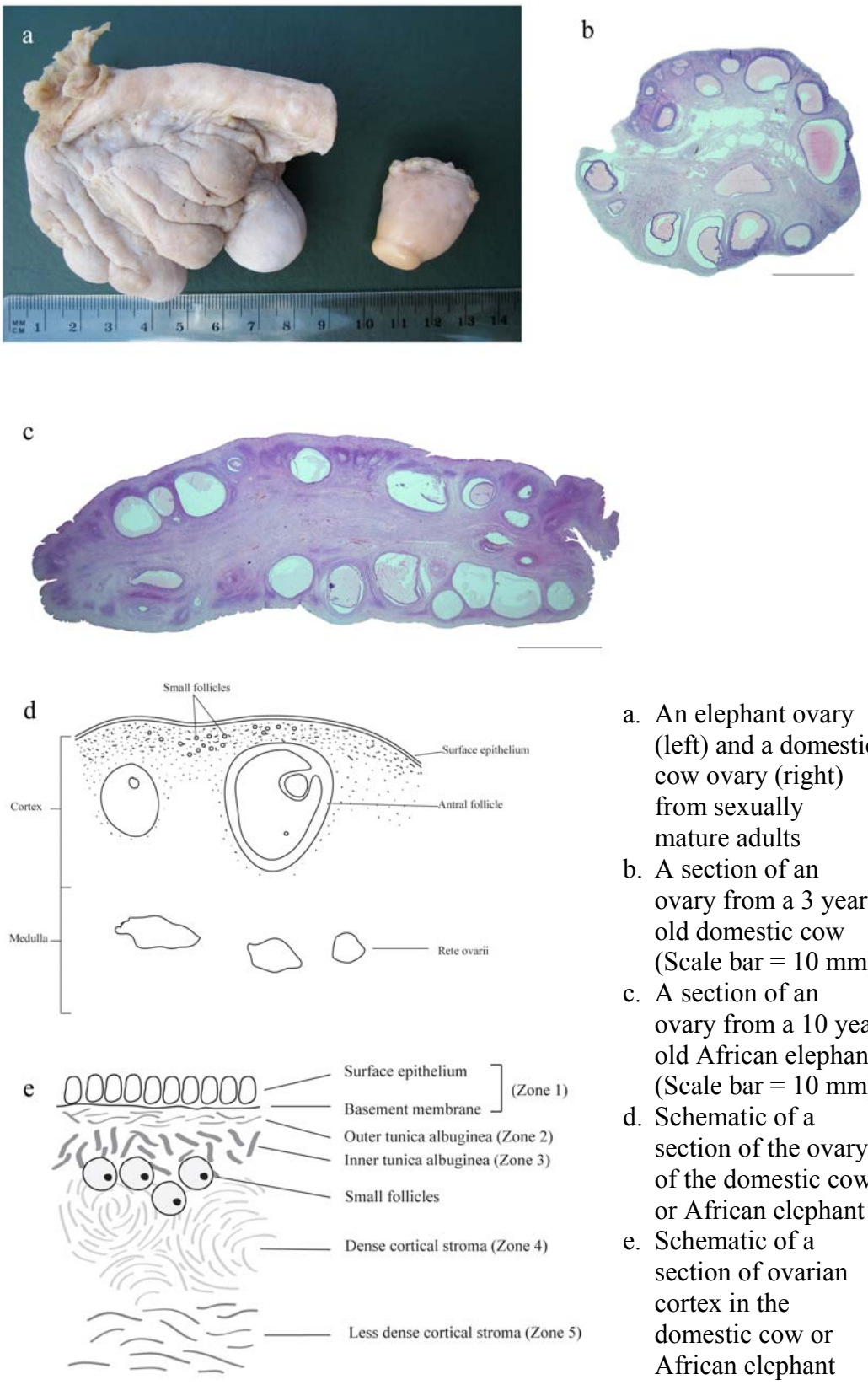
Zone 2; The outer region of the *tunica albuginea*. This is connective tissue composed of spindle-shaped fibrocytes and collagen fibrils generally lying parallel to the epithelium.

Zone 3; The inner region of the *tunica albuginea*. This contains collagen fibrils and also more rounded fibrocytes that are either randomly orientated or are arranged at right angles to the ovarian surface epithelium. The widths of Zone 2 and 3 are extremely variable and Zone 3 is sometimes non-existent. Zones 1–3 have very little blood supply.

Zone 4; This contains large quantities of collagen fibrils, similar to those seen in Zones 2 and 3, but with a much greater density of cells arranged in whorls. Large numbers of primordial and primary follicles are located in the outer region of Zone 4 and at the interface between Zones 3 and 4. Some secondary follicles and small antral follicles are also located in Zone 4 but are often deeper in the ovary than the primordial follicles.

Zone 5; The stromal cells here are less tightly packed and they exhibit a less dramatic whorl arrangement. Large antral follicles may be found here.

The boundary between the cortex and medulla of the ovary is poorly defined. The medulla consists of connective tissue through which a large number of contorted blood and lymph vessels protrude, bunching to form the hilus at the point where they exit the ovary to enter the mesovarial ligament. Preliminary studies indicate that a similar zonal layout to the bovine is present in the elephant ovary (Stansfield 2006).



a. An elephant ovary (left) and a domestic cow ovary (right) from sexually mature adults
b. A section of an ovary from a 3 year old domestic cow (Scale bar = 10 mm)
c. A section of an ovary from a 10 year old African elephant (Scale bar = 10 mm)
d. Schematic of a section of the ovary of the domestic cow or African elephant
e. Schematic of a section of ovarian cortex in the domestic cow or African elephant

Figure 1.6 Similar morphology of the ovarian cortex in the domestic cow and the African elephant

1.5.2. The ovarian reserve and follicle activation

1.5.2.1 Established dogma on the ovarian reserve

It is generally accepted that, in mammals, the resting pool of true primordial follicles containing all the oocytes required during reproductive life (Picton 2001) is formed during fetal life and the follicles are present in the ovary at birth (Gosden 2004; Zuckerman 1951). In cattle, sheep and pigs, follicles are reported to be randomly distributed in the cortex while in dogs and cats they occur in clusters (McGeady *et al*. 2006). Distribution studies need to be carried out to determine the spread of the small follicles within the elephant ovary.

Around 50 years ago the dogma evolved that the process of oogenesis in animals follows a uniform pattern, of which there are two main variants (Bukovsky *et al*. 2005):

i. Oogenesis appears to continue, either continually or cyclically, throughout reproductive life in most teleosts, all amphibians, most reptiles and, conceivably, a few mammals (Bukovsky *et al*. 2005). In lemurs, for example, oogonia persist into adult life (Gosden 1995).

ii. Oogenesis takes place only in fetal- or neonatal gonads, and oogonia neither persist nor divide mitotically during sexual maturity as in cyclostomes, elasmobranch's, a few teleosts, some reptiles, all birds, monotremes and, with a few possible exceptions, all eutherian mammals (Franchi *et al*. 1962).

More recently, however, this concept has been challenged by the immunocytochemical localization of so-called germ line stem cells (GSC) in the epithelial layer covering the outer surface of the ovaries (OSE) of adult mice (Johnson *et al*. 2004) and women (Tilly & Telfer 2009; White *et al*. 2012), and even the birth of live offspring from irradiated mice following transplantation of bone-marrow derived mesenchymal stem cells (MSC) into their oocyte-depleted ovaries (Zou *et al*. 2009). The argument still rages as to whether any post-natal oogenesis which may or may not occur in mice and women could originate from GSC within the ovarian cortex or must derive from migrating MSC (de Felici 2010).

1.5.2.2 Follicle morphology

Descriptions of the morphology of small ovarian pre-antral follicles or small follicles (SF) have been made in several species (Fortune 1994; Gougeon 1996; Hirshfield 1991; Peters 1969; see Table 1.12). From these and other studies it is generally accepted that follicles can be classified in a similar way throughout the range of mammalian species by examination of the shape, size, number and arrangement of the granulosa cells surrounding the oocyte (Griffin *et al*. 2006). For this study the description given by Oktay (1995) and Pepling (2012) was used (Table 1.13) and expanded as described in Section 1.5.4.

Table 1.12
Small follicle classification in mammals

Authors	Species	Description
Block (1951)	Human	Primordial: "Egg cell" surrounded by a single layer of flattened follicular cells. Situated in the outer zone of the ovary. Diameter seldom exceeds 40 µm Growing: Have a distinct cubical epithelium around the egg cell. Diameter >50 µm.
Lintern-Moore *et al*. (1974); Gougeon and Chainy (1987)	Human	B: Flattened granulosa cells B/C: Mixture of flat and cuboidal granulosa cells C: One layer of cuboidal granulosa cells
Richardson *et al*. (1987)	Human	Primordial: An oocyte surrounded by a single layer of flattened granulosa cells Primary: An oocyte surrounded by one or more layers of cuboidal granulosa cells. Follicles transitional between primordial/primary were classified as primordial.
Oktay (1995)	Rat	Primordial: Partly or completely encapsulated by squamous granulosa cells Primary: Single layer of cuboidal granulosa cells
Faddy and Gosden (1995)	Human	Stage1: Small unilaminar follicles one layer of squamous granulosa cells Stage 2: Unilaminar follicles, 1 layer of cuboidal granulosa cells or some squamous cells Stage 3: Growing follicle with enlarged oocyte and 2 or more layers of granulosa cells.
Wandji *et al*. (1996)	Bovine	Primordial: One layer of flattened somatic cells around the oocyte Primary: A single layer of cuboidal granulosa cells around the oocyte

Continued

Table 1.12 (Continued)

Van Wezel & Rodgers (1996)	Bovine	GC: Development cannot be relied upon to indicate the development of the follicle.
Wandji *et al*. (1997)	Baboon	Primordial: One layer of flattened somatic cells around the oocyte Early Primary: Unilaminar with both flattened and cuboidal cells Primary: One layer of completely cuboidal cells.
Lundy *et al*. (1999)	Sheep	Type 1: One layer of flattened granulosa cells. Type 1a – mixed flat and cuboidal granulosa cells Type 2: One to two layers of cuboidal granulosa cells
Cui and Yu (1999)	Yak	Primordial: One layer of granulosa cells Primary: Two layers of granulosa cells
Wright *et al*. (1999)	Human	Primordial: One layer of a majority of flattened pre-granulosa cells Primary: One layer of a majority of expanded cuboidal granulosa cells.
Meredith *et al*. (2000)	Rat	B: Flattened granulosa cells. B/C: Mixture of flat and cuboidal granulosa cells, C: One layer of cuboidal granulosa cells
Barber (2001)	Elephant	Primordial oocytes are surrounded by a single layer of squamous granulosa cells. Follicles become primary as the single layer of squamous cells differentiates into cuboidal cells.
Nichols *et al*. (2005)	Rhesus monkey	Primordial: Flattened granulosa cells Primary: One or several layers of granulosa cells, cuboidal or low columnar

Table 1.13
Small follicle classification (SF)

Type of follicle	Description
True Primordial (TPM)	Oocyte partially or completely encapsulated by squamous granulosa cells
Early Primary (EP)	At least one of the granulosa cells is observed to be columnar (enlarged)[a,]
True Primary (TP)	All the cells in the single layer of granulosa cells show enlargement.

[a] Expansion of the granulosa cells does not occur simultaneously and follicles may be viewed in histological sections which comprise one or more cuboidal cells but still also have some flattened granulosa cells. These follicles have been called "Early Primary" to indicate that some growth has started but they are not yet "True Primary" follicles.

1.5.2.3 Follicle activation

The molecular mechanisms that lead to follicle growth have not yet been established (McLaughlin & McIver 2009) but progress is being made (Reddy *et al*. 2010); they are thought not to depend on gonadotrophic hormones (Oktem & Urman 2010). It is generally understood that the first sign of activation that is visible with the light microscope is a change in the shape of the granulosa cells, from flattened to cuboidal, possibly under stimulation by factors from the oocyte (Rodgers & Irving-Rodgers 2010). Once the oocyte is surrounded by cuboidal granulosa cells it is termed a true primary follicle (TP). Hence, the classification of early follicle growth is rather subjective. The size of the follicles in the resting pool, and the size of primary follicles (TP) are given in Table 1.14 and Table 1.15.

The established theory that only true primordial follicles (TPM) make up the follicle reserve in mammals is based on the premise that follicles arrest with only squamous granulosa cells surrounding the oocyte and any subsequent deviation from this state indicates a commitment to growth which results in the follicles exiting from the reserve (Oktay 1995). However, significantly large numbers of early primary (EP) follicles have been observed in mammalian ovaries, including the elephant (Stansfield 2006), suggesting they may also form part of the ovarian reserve. This assumption is being

reassessed and is summarized well by Gougeon (2010). In healthy young women it is speculated that only one third of all small follicles are at the TPM stage (de Bruin *et al*. 2002), most are at the EP stage and a sizeable proportion have already reached the TP stage. In bovine ovaries it is also suggested that more than 80% of follicles are at the EP or TP stages (Van Wezel & Rodgers 1996). Likewise, Types "B and C" follicles (early primary) comprise 72–80% of the total population of small follicles in mature rats (Meredith *et al*. 2000). In contrast, many papers describe species in which the majority of follicles in the ovary at any one time are primordial (Braw-Tal & Yossefi 1997; Fortune *et al*. 2000; Hirshfield 1989; Oktay 1995; Picton 2001; Wandji *et al*. 1996).

Table 1.14
Diameter of follicles in the resting pool in various mammalian species

	Size of structures (µm)			
	Follicle	Oocyte	Nucleus	Granulosa cells in largest cross section
Elephant TPM[a]	36.1 ± 4.5	30.0	14.5 ± 2.7	5.2 ± 1.3
Elephant EP[a]	38.1 ± 3.9	30.0	13.9 ± 2.5	9.8 ± 1.3
Yak (mature)[b]	40–45		12.5–16	
Bovine				
Bos taurus[c]	<40	29.7		<10 flattened
Bos taurus[d]	35	25		
Bos taurus[e]	30–50	20–35		
Bos taurus[f]	28.9–38	26.8–31.2		
Bos indicus[g]	36.0	28.1		7.3
Human[h]	44	36	19.4 ± 8.5	
Pig[h]	34	29.1		
Hamster[h]	26	23.4		
Mouse[h]	17	12.6		

[a] (Stansfield 2006), [b] (Cui & Yu 1999), [c] (Fortune 2003), [d] (Fair 2003), [e] (van den Hurk *et al*. 1997), [f] (Van Wezel & Rodgers 1996), [g] (Kacinskis *et al*. 2005), [h] (Griffin *et al*. 2006), [i] (Forabosco *et al*. 1991)

Table 1.15
Diameters of various parts of primary follicles (μm)

	Whole follicle	Oocyte	Nucleus	Granulosa cells
Elephant[a]	46.9 ± 4.7	35.0	15.0	15.8 ± 2.8
Bovine				
Bos taurus[c]	40–80	31.1		
Bos taurus[d]	45	30		
Bos taurus[e]	40–60	30–40		
Bos taurus[f]				
Bos indicus[g]	48.5	31.7		14.6
Human [h]	70	42.1		
Pig [h]	64.9	37.4		
Hamster	56	32.2		
Mouse	52.1	28.8		

[a] (Stansfield 2006), [b] (Cui & Yu 1999), [c] (Fortune 2003), [d] (Fair 2003), [e] (van den Hurk *et al*. 1997), [f] (Van Wezel & Rodgers 1996), [g] (Kacinskis *et al*. 2005), [h] (Griffin *et al*. 2006), [i] (Forabosco *et al*. 1991)

1.5.2.4 Oocyte growth

Evidence suggests that oocyte growth follows, rather than precedes, the changes in the granulosa cells (compare the data in Table 1.15 with those in Table 1.16). The analysis of the correlation between oocyte diameter and number of granulosa cells during early growth of the follicle in the domestic cow reveals two distinct and consecutive stages (Braw-Tal 2002). Bovine primordial follicles contain an average of 6 granulosa cells in their largest cross section (Braw-Tal & Yossefi 1997) and a change in the shape of granulosa cells, from flattened to cuboidal, starts when there are at least 7 cells in the largest cross-section or a complete layer of around 20 cuboidal granulosa cells surround the oocyte (Hulshof *et al*. 1992). This is not accompanied by an increase in oocyte diameter. Very similar kinetics of granulosa cell transformation to those in the domestic cow have been reported for humans (Braw-Tal 2002).

Furthermore, there is a positive and linear correlation between the number of granulosa cells and oocyte diameter. The first significant change in bovine oocyte diameter is observed in follicles with at least 40 granulosa cells in the largest cross section (4th generation of granulosa cells according to Braw-Tal, 2002). In mice and rats the oocyte starts to grow earlier when there are about 10, mostly cuboidal, granulosa cells in the largest cross-section (2nd generation; Lintern-Moore & Moore 1979). In the human oocyte growth commences at the third generation (Braw-Tal 2002). Comparing the mouse, hamster, pig and human, Griffin *et al*. (2006) found that although the development of the follicle and oocyte was similar across species, the change in the ratio of follicle to oocyte diameter and the proliferation of granulosa cells were all shown to be species specific.

1.5.2.5 Zona pellucida

From immunocontraceptive studies it has been established that the cellular origin and temporal formation of the zona pellucida is species-specific (Fayrer-Hosken 2008). Upon follicle activation a zona pellucida is secreted between the growing oocyte and the granulosa cells and it becomes evident during the primary follicle stage in the rat (Odor 1960), mouse (Wassarman & Josefowicz 1978), rhesus monkey (Zamboni 1974), pig (Sinowatz *et al*. 1995) and cow (Russe 1983). Similarly, contraceptive studies have shown that the zona pellucida also appears during the primary follicle stage in the elephant (Barber & Fayrer-Hosken 2000; Fayrer-Hosken *et al*. 1999).

1.5.3. Continued follicle growth to pre-ovulatory size

At around the secondary follicle stage FSH receptors develop on the granulosa cells and stimulation results in granulosa cell proliferation (Gougeon 1996). At the pre-antral multilayer stage a layer of spindle-shaped stromal cells is recruited outside the basal lamina of the follicle and this forms the *theca interna* which develops LH receptors (Johnson & Everitt 2004). This is usually the maximum degree of development found in the pre-pubertal ovary (Genuth 2004). Gonadotrophin stimulation at puberty results in the production of steroids from both the granulosa cells (oestrogens) and theca cells (androgens). Without such stimulation the follicle will undergo atresia (Gougeon 1996). Granulosa cells also start to exude antral fluid into vesicles which unite to form the antrum of the follicle (Peters & McNatty 1980). With further enlargement cells from the

stroma and an in-growth of blood vessels constitute the theca externa which allows faster access of blood-borne gonadotrophins to the inner parts of the follicle (Young 2010). The antral follicle now stands at approximately 2–5 mm diameter in the human (Genuth 2004).

During the final stage of follicle growth a whole cohort of follicles become "selected" and they increase their diameter by further enlargement of the antrum (follicle diameter 7–10 mm). Eventually, in monotocous species such as the elephant, one follicle becomes dominant and it continues to grow to the point of ovulation (20–25 mm) while the remainder of the original cohort undergo atresia (Findlay *et al*. 2001). During follicle development in cattle the oocyte is extremely active metabolically such that, from primordial to preovulatory size, it is estimated to increase 204-fold in volume (Braw-Tal & Rodgers 1996).

1.5.4. Follicle classification

Considering the widely agreed terminology used in the literature quoted above, the following classifications will be used to define the follicle populations described in the present study:

1.5.4.1 True Primordial follicles

Primordial follicles comprise an oocyte, arrested in the dictyate stage of meiosis I, surrounded by a low number of squamous granulosa cells and enclosed by a basement membrane. A useful definition of a squamous cell is that its height is negligible in comparison to its other dimensions so that it appears as a slender rod which is slightly thickened in the vicinity of its oval nucleus (Bloom & Fawcett 1962). Thus, using this description, a cell that is thicker than the diameter of its nucleus can be described as expanding.

1.5.4.2 Early Primary follicles

According to the definition used for bovine follicles (Van Wezel & Rodgers 1996), some of the granulosa cells of the EP follicle have expanded and become cuboidal, often making the follicle more prolate in shape with a mean of maximum-to-minimum diameter ratio of 1.33 μm. The prolate shape is attributable to a clustering of granulosa cells at the

two opposite poles on the long axis of the follicle (Braw-Tal & Rodgers 1996). For a comparison of EP follicles among various species see Table 1.15.

1.5.4.3 True Primary follicles

TP follicles consist of primary oocytes surrounded by a single layer of cuboidal granulosa cells which begin to express markers of cell proliferation such as proliferating cell nuclear antigen (Wandji *et al*. 1996). The size of TP follicles in a range of species is given in Table 1.15.

1.5.4.4 Secondary follicles

Progression to the secondary follicle stage is characterized by the appearance of a second layer of granulosa cells (Driancourt 1991) which, in the cow, increases to 6 layers when the follicle reaches a diameter of around 150 µm. During the early growth of the secondary follicle, connective tissue fibres become arranged parallel and peripheral to the basement membrane — which encloses the granulosa layer — to form the thecal layer. By the end of the secondary stage, the thecal layer becomes dominated by large, epithelioid, hormone secreting cells and a capillary network (van den Hurk *et al*. 1997). At the same time cortical granules become visible within the oocyte cytoplasm (Fair *et al*. 1997). In *Bos taurus* the diameter of the oocyte is approximately 45–60 µm (van den Hurk *et al*. 1997) and in *Bos indicus* at a follicle diameter of 88.4 µm the oocyte has a diameter of 43.8 µm with approximately 62 granulosa cells in the largest cross-section (Kacinskis *et al*. 2005). Cui and Yu (1999) reported similar measurements in the Yak with follicle diameters of 80–120 µm, oocyte diameters of 40–45 µm and the nucleus of the oocyte at 15 µm.

1.5.4.5 Small preantral follicles

Production of a range of molecules by the granulosa cells creates an osmotic gradient which draws fluid into the follicle across the basement membrane from the thecal vasculature. This creates further expansion of the follicle and considerable remodelling of the granulosa cell junctions is also required to create the follicular antrum filled with follicular fluid (Rodgers & Irving-Rodgers 2010). In cattle, the diameter of small preantral follicles ranges from 81 to 130 µm while the mean oocyte diameter measures

approximately 49.5 µm (Fortune 2003). See Table 1.16 for a comparison of preantral follicles among various species.

Table 1.16
Diameter of various parts of preantral follicles (µm)

	Follicle	Oocyte	Nucleus
Yak [a]	145–180	60–80	25
Bovine			
Bos taurus[b]	131–250	68.6	
Bos taurus[c]	300	65	
Bos taurus[d]	150	60	
Human[e]		80	
Pig[e]	300	90	

[a] (Cui & Yu 1999), [b] (Fortune 2003), [c] (Fair 2003), [d] (van den Hurk *et al*. 1997), [e] (Griffin *et al*. 2006).

1.5.4.6 Small antral follicles

The extent to which the antrum of the follicle expands differs between dominant and subordinate follicles, and among species, with larger animals tending to have larger follicles consisting of 95% follicular fluid (Rodgers & Irving-Rodgers 2010). In cattle, follicles at the mid antral stage have diameters of 3 mm and oocytes 110 µm (Fair 2003). In Yak, follicles of 300–500 µm in diameter would have one complete antrum with oocytes of 60–90 µm and oocyte nuclei of 35–40 µm diameter (Cui & Yu 1999). Oocyte diameter in small antral follicles of 2.5–5.5 mm diameter in elephants has been measured at 155–165 µm (Stansfield 2006).

1.5.4.7 Graafian follicles

A Graafian follicle (or tertiary follicle) is the term used to denote one of a group of growing selectable follicles which may or may not ovulate (Gosden & Lee 2010) whereas an ovulatory follicle is one that has acquired LH receptors on its mural granulosa cells and is destined to ovulate in response to increased levels of pituitary LH (Gougeon 2010).

In cattle the ovulatory follicle reaches a diameter of 15–20 mm and the oocyte 120–130 µm (Fair 2003). Similarly, in women oocytes reach approximately 120 µm in diameter at the time of ovulation (Gosden 2005). Ultrasound studies on Asian and African elephants report the diameter of the preovulatory follicle as 20–21 mm (Hermes 2000; Lueders *et al*. 2010). This is somewhat bigger than the maximum diameter of 13.7 ± 0.7 mm noted in the elephant during the first LH surge early in the interluteal period (Hermes 2000).

1.5.4.8 Atretic follicles

In all mammalian species studied to date the store of primordial follicles decreases between birth and puberty (Gougeon *et al*. 1994). Although follicles start to grow during this period they do not reach ovulatory size due to inadequate gonadotrophic stimulation (Gosden 1995). In sheep, comparison of the populations of primordial follicles in the ovaries of 2- and 8-year-old ewes has demonstrated that 8 primordial follicles disappear from the reserve pool every day between these ages (Driancourt *et al*. 1985). This contrasts with the estimated rate of initiation of growth from the follicle pool (2–3 follicles per day) and suggests that 5–6 follicles die every day within the primordial pool; in cattle this figure is estimated to be 6 per day (van den Hurk & Zhao 2005). Since granulosa cell apoptosis is never visualized in such follicles, it may be assumed that death of the oocyte is the cause of this high rate of loss of primordial follicles. Oocyte death appears to be very fast and pycnotic bodies, the most common marker used to identify a dying cell, are seldom visualized in oocytes (Reynaud & Driancourt 2000).

1.5.5. Small follicles in elephant ovaries

During a pilot study to the present project, Stansfield (2006) noted that non-pregnant adult elephants appeared to be endowed with a relatively low number of small primordial follicles, such that TPM follicles showing no expansion of the granulosa cells numbered less than 0.6% of the total SF population. The majority of SF were present as EP (56–80% of all SF) and TP (19–44% of SF). It was concluded that elephants do not store their oocytes in a true “resting follicle pool” and that constituent follicles have variously developed granulosa cells which range from flat to cuboidal in outline. This point clearly required further investigation and was therefore addressed in the present study reported in this thesis.

1.6. Background to the over-abundance of elephants in Zimbabwe

1.6.1. Elephant numbers in Zimbabwe

Historically, man has reduced elephant numbers. This was recorded as long ago as AD 77 when Pliny noted that the elephant herds in North Africa had been wiped out (Meredith 2001). Furthermore, along with slaves and gold, ivory has, at times, been Africa's major export (Carruthers 2008). Natural curbs on elephant population growth include drought, which kills off particularly the young and the old, lion predation on calves and diseases such as anthrax, which was once wide-spread in Zimbabwe (Hugh-Jones & de Vos 2002). Provision of artificial water sources, a reduction in lion populations due to hunting and human encroachment and effective control of anthrax has meant that elephants nowadays have few natural population control mechanisms during years of average or above average rainfall.

Between 1980 and 2004 Zimbabwe's elephant population increased at a rate of approximately 5% per annum (Table 1.17). If this growth rate has continued since 2004 the present population could number around 120 000. The area of land available for elephants to roam over is 63 784 km^2. This equates to around 2 elephant per km^2 which is well in excess of the widely recommended figure of 0.5–0.6 elephants per km^2 (Foggin 2003). In an effort to control this booming elephant population cropping by shooting was instituted in 1960 and by 1988, some 44 000 elephants, mostly whole breeding families, had been culled. Following cessation of culling activities, between 1989 and 1995, around 2500 more elephants were shot during problem animal control (PAC) operations.

Table 1.17
Estimated elephant numbers in Zimbabwe

Year	Population size	Number culled	Author
1871–1875	"Vast numbers"		Bryden (1889)
1900	4 000		Cumming & Jones (2005)
1930	10 000		Child (2004)
1940	17 000		Child (2004)
1950	25 000		Child (2004)
1980	47 000		Nduku (1991)
1960–1991		46 775	Child (2004)
1991	70 000		Nduku (1991)
2001	88 100		Child (2004)
2004	100 000		Cumming & Jones (2005)
2010	120 000?		

Ecologically, elephants are a keystone species. At densities below 0.25 elephants per km^2 their impact on habitat is limited. Between 0.25 and 1.0 elephants per km^2 structural change occurs in woodland with the result that there are fewer mature trees (Figure 1.7). When the density becomes higher than one elephant per km^2 woodland may be converted into treeless grassland or shrubby coppice states with resulting impacts on the habitats of other herbivores (Kerley & Landman 2006).

The human population of Zimbabwe is also expanding and people are moving into areas previously used by elephants many of which are contiguous with Reserves or National Parks. The value attributed to an elephant depends on the socio-economic background of the observer (Rosa & Joubert 2009). To a tourist on safari an elephant may be a majestic, sentient creature which has as much right to occupy space on planet Earth as members of the human race. To a rural African an elephant may variously be viewed as a source of meat, a threat to his crops and livestock or even to his own life. Hence, many rural Africans regard the elephant as open access property which provides little or no benefit and is a direct competitor. This leads to human-elephant conflicts (HEC) which include crop raiding, granary destruction and loss of both human and elephant lives (Nelson *et al*.

2003). Between 2002 and 2006 more than 5000 cases of HEC were recorded in Zimbabwe which resulted in the killing of 774 elephants during subsequent PAC operations (Campfire 2007). With the populations of humans and elephants both continuing to increase the incidence of HEC can be expected to rise in future years.

In Tsavo National Park, Kenya, some 10 000 elephants—approximately 25% of the population—died of thirst and starvation during a severe drought in 1970-1971 (Meredith 2001). In 2009 a similar situation occurred in Amboseli National Park in Kenya when around 300 elephant died of starvation (Lee 2010) along with 83% of the wildebeest (18 538 reduced to 3098) and 71% of the zebra (15 328 reduced to 4432) populations (Burnham & Gronewold 2010). With the increasing shortage of available land in southern Africa, elephants are being forced into smaller range areas and they can no longer move around the continent, or even the country, as they once did (Whyte 2002). As a first step towards their active management in Zimbabwe boundaries were placed around elephants. If a diverse ecosystem is to be maintained within those defined locations, it is beholden upon man to control expansions of the elephant population that threaten the viability and diversity of that ecosystem. The tragedies in Tsavo and Amboseli show that taking a *laissez faire* attitude will inevitably lead to elephants dominating and changing the ecosystem (even if this may be temporary) to the detriment of other species of both fauna and flora and, eventually, to the elephants themselves. If biodiversity is a priority in Game Parks steps need to be taken to limit the negative impact of elephants on their environment, preferably with the support of valid and current research efforts (Marais & Hadaway 2006).

Figure 1.7 Damage to trees caused by elephants in Savé Valley Conservancy and Mana Pools National Park, Zimbabwe

1.6.2. Attempts to counteract over-abundance

Lotter *et al*. (2008) recorded that "In an ideal world all humans would treat elephants in ways that appropriately acknowledge and respect their moral standing. Elephants would have enough land available to freely live their lives as they see fit and to migrate to other areas when they deem it appropriate. In such a world humans would have no reason to intervene in their lives. However, we do not live in such a world. As a result of the violent history between our species, the exponential growth in human population, and the resultant loss of elephant habitat, conservationists must explore various management options to create the best life possible for elephants within current constraints".

The following management "tools" may be used, alone or in conjunction, depending upon the prevailing aims and views.

1.6.2.1 Passive management options

Laissez-faire: A "nature knows best" approach may be appropriate where ranges are large enough; ie larger than 100 000 km^2 (Cumming & Jones 2005) and elephant populations are self regulating. It is favoured by many on moral and ethical grounds as it is considered non-lethal in terms of human-induced mortality. However, when elephant populations do exceed their available resources the stress and suffering they undergo during drought-induced starvation and mass die-offs may be considerable, inhumane and ethically reprehensible.

Metapopulations: Source-sink migration involves the natural movement of animals to areas of lower population density which may be encouraged by closing down artificial water holes in the high density areas. Difficulties occur when such wildlife areas are surrounded by rural communities. This disrupts the free movement of the elephant through the inhabited land and stimulates HEC leading to PAC. Such dispersal of elephants would inevitably occur slowly as they need to move under their own momentum (Slotow 2005) and considerable community co-operation would be needed to implement such movements.

Fencing elephants out: This may be applicable on a small basis but elephant proof fencing is expensive and not affordable by small rural communities. Biological

alternatives such as bee hives (King *et al*. 2007) and the use of chilli peppers in various forms (Osborn & Parker 2003) may be suitable in some circumstances.

1.6.2.2 Active management options

Translocation: The movement of elephants in whole family groups is considered the least stressful and most successful means of relocation. Past movements of only juvenile animals have resulted in them showing aberrant behaviour towards other species in their new environment (Slotow 2001). Translocated elephants are heavily stressed during the capture and transport processes and they need considerable time to adjust to their new environment (Viljoen 2008). If the animals that remain in the source area also suffer stress, as has been occasionally noted, this will happen whether the missing elephants have been translocated or culled (Scholes 2007). Additionally, the distance moved needs to be substantial to prevent the translocated elephants returning to their origin, as was experienced in Kruger National Park in South Africa (Hofmeyr 2004). With a relocation distance of 500 km, for example, the cost of translocating one elephant was estimated to be US$ 8700 while the cost of translocating 100 elephant reduced to approximately US$ 1000 per elephant (Grobler *et al*. 2008). However, the biggest problem with translocation nowadays is that there is no longer any requirement for additional elephants in any game parks or conservancies within Zimbabwe, South Africa, Botswana or Namibia.

Culling: Culling results in an immediate reduction of the numbers of a population and it reduces the pressure on the ecosystem of the National Park or conservancy. Stress for the culled animals is very brief and that experienced by any remaining associated family groups is no more than when translocation has taken place (Whyte 2001; Whyte 2002). Furthermore, the sale of elephant products from culls (meat, hides, tusks etc) results in the sustainable usage of an elephant population by ranchers and the local rural populations (Lotter *et al*. 2008). The latter need to participate in decisions and benefit from the presence of the elephants on their land, otherwise they view the elephant as direct competition and desire their removal. Culling may however result in a stimulation of the rate of reproduction due to its impact of reducing elephant population density (Laws et al. 1975). More recent studies cite such increases as a response to the complex interaction of resource availability (Caughley 1983; Chamaille-James et al. 2008) rather than specifically due to population density.

Elephants that raid crops do tend to originate from bachelor herds which potentially carry a higher value as trophy animals (Cumming & Jones 2005); therefore problem animal control has serious economic and socio-economic implications which affect both peoples' livelihoods and the financial viability of wildlife management within conservancies, especially with respect to the trophy hunting of elephants. Implementation of a culling programme is an emotive and difficult decision to take but, given the present economic circumstances within Zimbabwe and the aims of maintaining biodiversity within National Parks and conservancies, there is currently no alternative (Foggin 2003).

Reproductive Strategies: Unless the reproductive rate of elephants can be reduced, other tools such as culling or translocation will need to be practiced continually. To this end reproductive strategies are sought to slow down population growth rates. The most promising to date is immunocontraception (Bertschinger *et al*. 2008). Elephant oocytes, along with those of at least 100 other mammalian species, including horses (Kirkpatrick *et al*. 1990), possums (Cowan *et al*. 2003) and white-tailed deer (Kirkpatrick *et al*. 1997), share zona pellucida epitopes with the pig. For this reason, porcine zona pellucida (pZP) proteins have been tested and show the most promise so far as the basis of a vaccine to attempt to limit elephant reproduction by blocking fertilization (Fayrer-Hosken *et al*. 1999). The largest elephant herds in which contraception has so far been successful numbers only 80 head (Delsink *et al*. 2007) and the logistics and cost of the operation, coupled with the need for repeated booster injections, currently prevent its use on larger populations. For example, in Zimbabwe at least 75% of all the breeding females would need to be rendered infertile for at least 10 years to produce a significant drop in the overall population (Foggin 2003). Additionally, contraception does not result in an immediate reduction in population size and a considerable time delay would occur before female elephants that were given the contraceptive treatment would start to die from old age and thereby commence the desired population reduction (Stout & Colenbrander 2004). Following immunocontraception calves would continue to be born for a period of 2 years after the first vaccination since immunisation does not interfere with an existing pregnancy (Delsink *et al*. 2007). Some side effects from the use of this anti-pZP vaccine have been observed (Grootenhuis 2003; Paterson *et al*. 1992; Paterson *et al*. 1996). Although there is concern about the reversibility of the method after long-term usage, which is particularly pertinent for elephants, given their extended reproductive life (Perdok *et al*. 2007), two calves were born from elephant cows in a South African reserve

in 2011 following 7 years of treatment (Delsink 2011). A further major problem with its use in wild elephants is effective and practical delivery of the vaccine (Bertschinger *et al*. 2008).

Long term effects of the pZP vaccine on fertility and also the social effects of the absence of calves on the herd need to be considered. In addition, repeated 16-week oestrous cycles is not a natural reproductive state for the African elephant which would normally only experience one period of overt oestrus and mating every 4 years. The resulting unnatural state of mature bulls being more frequently in contact with female family groups could well disturb social structures and be particularly hazardous for young calves (Poole 1989). These are being addressed by suggestions of rotational treatment (Druce *et al*. 2011) Equally important, perhaps, the ethics of spending such a large amount of money to prevent a species from breeding when the funds could be better used to improve the welfare of the local protein-deficient human population must be borne in mind (Cumming & Jones 2005).

The possibility of the treatment of far fewer elephants by relying on the mate guarding behaviour of dominant bulls has stimulated research into male contraception using gonadotrophin releasing hormone vaccine or dominant bull sterilization (Bokhout et al. 2005).

Alternative reproductive strategies: An alternative strategy for potentially restricting the reproductive capacity of female elephants is to target the earliest stages of oocyte growth in the ovary; namely, the oocytes contained within small follicles (Stansfield *et al*. 2011b). Tens of thousands of primordial follicles populate the ovaries of mammals from which the fertile oocytes will be ovulated during each reproductive cycle. Thus, the number of primordial follicles in her ovaries ultimately determines any female animal's reproductive longevity. The means by which primordial follicles are held in their resting state or are stimulated to grow are not fully elucidated (Gleicher *et al*. 2011). While in this resting stage the follicles are endocrinologically inactive which, together with their residence within a relatively avascular part of the cortex (Oktem & Urman 2010), suggests a local follicular factor, either within the oocyte or in the granulosa cells keeping them inactive. Possible candidates could be oocyte genes, either for stimulating (eg. c-kit, TGF-β, AMH, GDF-9, Figα) or inhibiting (eg. retinoblastoma protein, Wilms tumor suppressor gene, follistatin (Picton 2001). In order to target contraceptive development

towards the early stages of follicle and oocyte development in the elephant it is first necessary to fully understand the concepts of reproductive ageing and ovarian reserves in this species.

The use of hunting and culling programmes as management tools to attempt to limit the growth of elephant populations within Conservancies and Safari Areas in Zimbabwe gave the unique opportunity to undertake the research on folliculogenesis in the elephants ovary which is described in this thesis.

1.7. Objectives of the study

There is a lack of information on the status and dynamics of small ovarian follicles in the African elephant. Such knowledge may be of use in studies targeting the ovary when attempting to achieve contraception in wild elephants and to determine whether the depletion of the ovarian reserve may be a cause of the observed poor fertility in captive zoo elephant. Such knowledge would also permit comparison of their ovarian reserve with that of women who display similar reproductive longevity.

The aim of this study was to define the ovarian follicular reserve of wild African elephants in terms of its type of SF, its establishment and distribution throughout the ovaries, and the change in numbers of SF in the embryo and fetus as well as throughout prepubertal and adult life.

More specifically, studies with the following aims, as reported in subsequent chapters of this thesis, were performed:-

i. To determine the nature of the SF constituting the follicle reserve in the African elephant

ii. To determine distribution of these SF in the ovaries.

iii. To study the development of the germinal ridge in the embryo along with the establishment of the SF reserve in early fetal life.

iv. To monitor the further growth and development of the elephant ovary from mid fetal life to prepuberty, along with its reserve of SF.

v. To quantify the SF reserve between puberty and reproductive senescence, and to determine the timing of such senescence.