Chapter 7. The progression of small follicle reserves in wild African elephants (*Loxodonta Africana*) from puberty to reproductive senescence

7.1. Introduction

The accepted dogma for the ovarian reserve in mammals is a steady loss of small follicles through natural attrition and ovulation during pre- and post-pubertal life (Gosden 1987), leading to full depletion and reproductive senescence in the majority of individuals that achieve their maximum lifespan (Cohen 2004).

Elephants are one of the longest lived land mammals and it is therefore of interest to study their longevity of reproduction. Behavioural studies (Moss & Lee 2011) and information from culling exercises (Freeman *et al*. 2008; Hanks 1972; Laws 1969) support the concept of a long reproductive life but there has been no research into the capacity of the ovary to supply viable oocytes throughout life. A useful comparison may be made with women in whom the process of reproductive ageing is determined by depletion of the ovarian pool of non-growing follicles (Hansen *et al*. 2008). Women exhibit peak fertility during their mid-twenties after which there is a general decline and a constantly increasing rate of loss of small follicles until menopause and the consequential cessation of fertility at an age of 51±8 years (Faddy & Gosden 1996), following which they may experience a considerable post-reproductive lifespan. The ability of the human oocyte to be able to maintain a state of meiotic arrest for up to 50 years has been praised (te Velde & Pearson 2002) although this may be surpassed by the elephant oocyte's ability of over 60 years (Moss & Lee 2011). It is also of interest to make a comparison of the ovarian reserve in wild elephants with that of captive elephants in zoos in Northern Hemisphere countries (when *post mortem* specimens become available), the fertility of which is severely compromised (Brown *et al*. 2004a).

The first aim of this study was to determine whether there is a progressive decline in the number of small follicles (SF) in the ovaries of African elephants from puberty through sexual maturity and into old age. The second was to determine whether a depletion of the follicular reserve poses a constraint on fertility of old African elephants.

7.2. Materials and methods

7.2.1. Animals

Thirty-one pairs of ovaries were obtained from post pubertal elephants during managerial off-takes within private conservancies and from professional hunting safaris in Zimbabwe. Only the ovaries of non-pregnant elephants (Figure 7.1b) were used in the study due to the distortion of the cortex caused by the development of multiple large corpora lutea during pregnancy (Figure 7.1a).

Nine of the elephants were killed during managerial off-takes. They were shot in family groups with no prior knowledge of the composition of the family groups. Criteria for selection of the family was a distinct grouping of 10 to 17 elephants within the Conservancy in an area of overabundant population or one of human-elephant conflict. All the members of the family group were shot with medium to heavy caliber rifles by professional hunters. Ovaries collected from hunting safaris (n = 21) were from tuskless females placed on hunting quota by the Parks and Wildlife Management Authority of Zimbabwe, in an attempt to reduce the number of tuskless elephant in the national population. One set of ovaries was collected from a semi-domesticated elephant that died of natural causes.

The animals were aged according to Laws (1966) and Lee (2012). Additional data was collected on lower jaw dentition and on shoulder height and back length of each animal for ageing purposes.

The elephants were divided among 5 age groups: Sub-adults (10–15 years), when most elephants are between puberty and first calving (Laws 1969); Young Prime Adults (16–25 years); Older Prime Adults (26–35 years); Senior (36–50 years) and Old Adults (51–70 years).

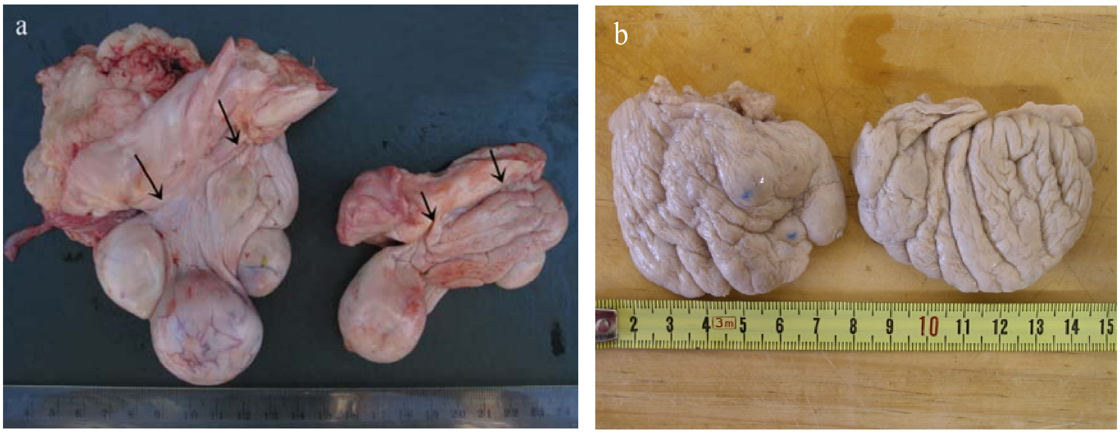

Figure 7.1 Ovaries from a pregnant and a non-pregnant African elephant cow

a. The two ovaries from a pregnant elephant cow (11 months gestation) showing the multiple corpora lutea of pregnancy, the ovary ipsilateral to the pregnancy is on the left. Arrows denote the attachment of the ovaries to the mesovarium.
b. The two sides of a bisected ovary from a non-pregnant cow.

7.2.2. Collection and processing of specimens

Collection and processing of the ovaries are described in Chapter 2. Briefly, the ovaries were removed from the carcass within 2 hours of death and fixed in 4% neutral buffered formalin. Subsequently, twenty 25 µm thick sections were cut from the ovaries of each animal using a uniform random sampling method (Howard & Reed 2005) and stained with haemotoxylin and eosin. A stereological protocol using Cavalieri's Estimator for volume and the unbiased brick for density calculations, as first described by Gundersen & Jensen (1987), were used.

7.2.3. Estimation of the age of *corpora nigra* (CN)

CN form as a result of degeneration of *corpora lutea* and remain in the ovaries for up to 77 months (Smith & Buss 1975). A sample group of 3 elephants aged 30–35 years old with CN in their ovaries were selected (some were pregnant and were not otherwise used in the current post-pubertal age group study) and the maximum size of CN in their ovaries measured. Age was defined as the interval between the origin of the CL that transformed into a CN and the time at which the ovaries were collected. Taking an elephant that was 21 months pregnant at the time of death as an example, the age of the cohort of CN that included the largest one was estimated as follows: Assuming that the cohort of CN remained from the beginning of the previous pregnancy (Hodges 1998; Stansfield & Allen 2012); knowing that gestation length is 22 months (Moss & Poole 1983). Assuming that the duration of post-partum anoestrus is 24 months (Laws 1969; Williamson 1976), and knowing that the elephant was 21 months pregnant at the time of death (Craig 1984), it follows that the estimated age of the cohort of CN is 22 + 24 + 21, which amounts to 67 months.

7.2.4. Statistical analysis

Where the data did not meet the requirements for parametric tests medians of more than two groups were compared with the Kruskal-Wallis test or, for two groups, with the Wilcoxon Rank-sum test. When data met the requirements for a parametric test, a t-test was used to compare two groups. After the Kruskal-Wallis test, the Kruskal-Wallis z-test with Bonferroni's adjustment was used to compare pairs of age groups. For all comparisons α was set at 0.05.

The relationship between the numbers of SF and age were analysed in two phases: In the first, the age groups as they were prospectively defined were compared. In the second, visual appraisal of the numbers of SF in elephant against age led to a post hoc comparison of the number of SF of older prime adults with young prime adults in order to determine whether the follicular reserve was lower in the older group.

The variance of the number of TP follicles among age groups was compared by means of the Modified-Levene-Equal variance test. The number of TP in elephant of the different age groups were compared by means of a Kruskal-Wallis test, followed by pairwise comparisons using the Kruskal-Wallis z-test with Bonferroni's adjustment.

The coefficient of variation for the number of SF counted in each of the two ovaries was determined (Dohoo *et al.* 2009). NCSS Statistical Software 2004 (NCSS, Kaysville, UT, USA) was used for statistical analysis.

7.3. Results

7.3.1. The relationship between the number of small follicles and age

Age affected the number of SF per elephant (P<0.001). Figure 7.2 suggests that the number of SF per elephant is more variable in sub-adults and young prime adults than in older elephant. Pairwise comparison among age groups show that sub-adults and young prime adults have higher numbers of small follicles per elephant than old elephants (Table 7.1). A post-hoc comparison between young prime adults and older prime adults revealed that the young prime adults had more SF than the old prime adults (P = 0.01).

Table 7.1
The number of small follicles (SF) per elephant of different age groups

		Number of small follicles		
Age group (years)	n	Median	25th Percentile	75th percentile
Subadults (10–15)	6	253 455[a]	171 142	556 949
Young prime adults (16–25)	6	386 974[a]	162 257	613 635
Older prime adults (26–35)	6	105 054[ab]	44 776	154 750
Senior adults (36–50)	6	57 920[ab]	45 133	79 307
Old (51–70)	7	11 113[b]	0	50 837

[a, b] Medians not sharing a common superscript differ (P < 0.05)

Table 7.2 shows the number of SF in each of the 7 old elephant in the study. Two of the old elephant had no SF whereas a third had none in one ovary and a mere 3020 in the other. Five of the 7 had fewer SF than each other elephant in the study except one 29-year-old elephant that had no SF in one ovary and 2392 in the other.

Excluding the 2 elephants that had no SF in either ovary and the 2 that had no SF in one ovary and a low number (2392 or 3020) in the other, the CV of the difference between the number of SF in the two ovaries of an individual was 11.1% (n = 27), showing that the numbers of SF in the two ovaries of an elephant were quite similar.

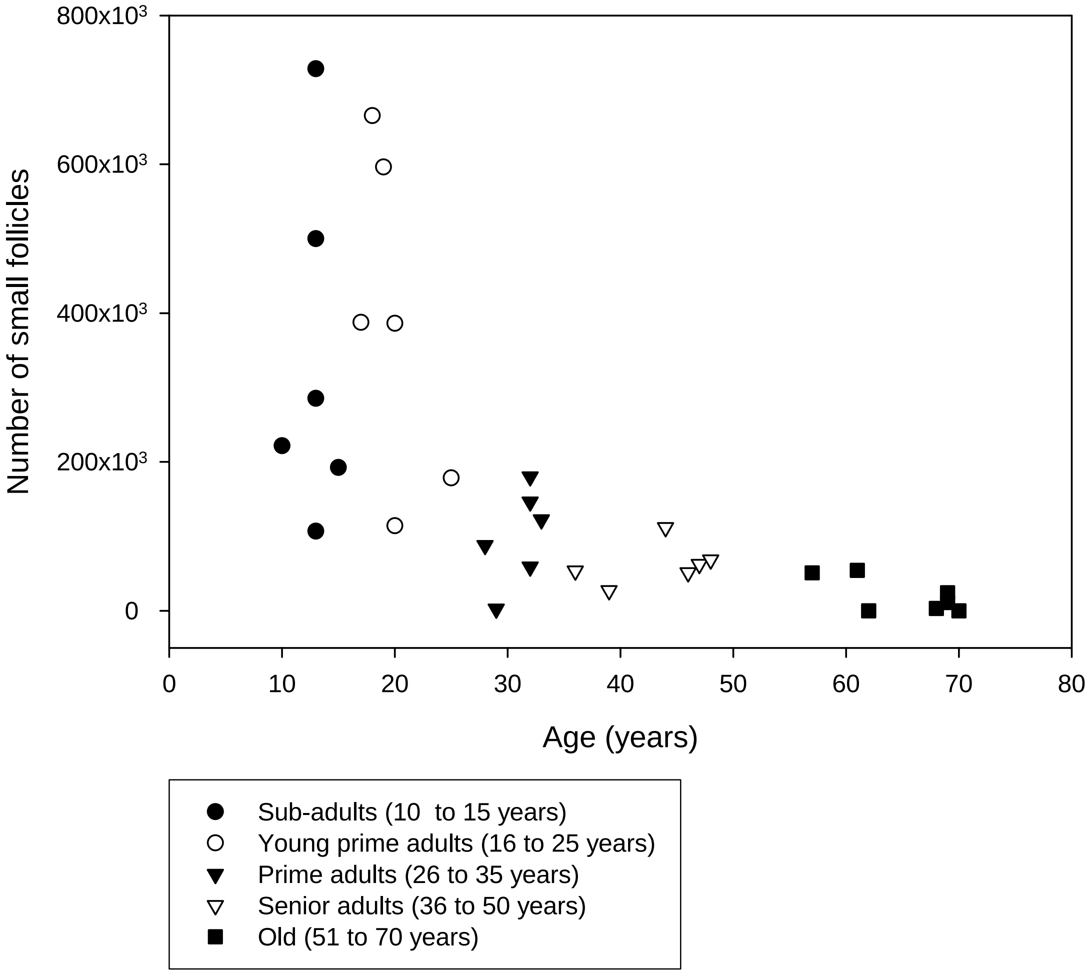

Figure 7.2 The total number of small ovarian follicles (SF) in elephant of different ages

Table 7.2
The number of small follicles (SF) in the two ovaries combined, as well as other signs of current or recent ovarian activity in 7 old African elephants

			Diameter of the largest structure (mm)[a]			
Elephant	Age (years)	Small follicles	Graafian follicle	Corpus luteum	Corpus nigrum	Lactation status
10–48	57	50 837	8		15	Lactating
19	61	54 337			11	Lactating
31	62	0	6		8	Lactating
54	68	3 020[b]			0	Lactating
09–14	69	24 278	6		15	Lactating
79	69	11 113	8		11	Lactating
64	70	0			5	Lactating

[a] An empty cell indicates that an elephant had no structure of the type that was visible with the naked eye
[b] All the small follicles occurred in one ovary, while the other had none

7.3.2. The relationship between the type of small follicles and age

Irrespective of their age, all but two elephants had 150 000 or fewer TP (Figure 7.3, Table 7.3) while EP follicle numbers were initially much higher than TP but became depleted by 45 years of age to leave only TP forming the follicle pool for the remainder of reproductive life.

The number of TP was similar among all age groups (median 46 770, 25th percentile 20 475, 75th percentile 89 120, $P = 0.18$), as was the variance ($P = 0.64$). The pairwise comparisons among groups revealed no differences ($P>0.05$).

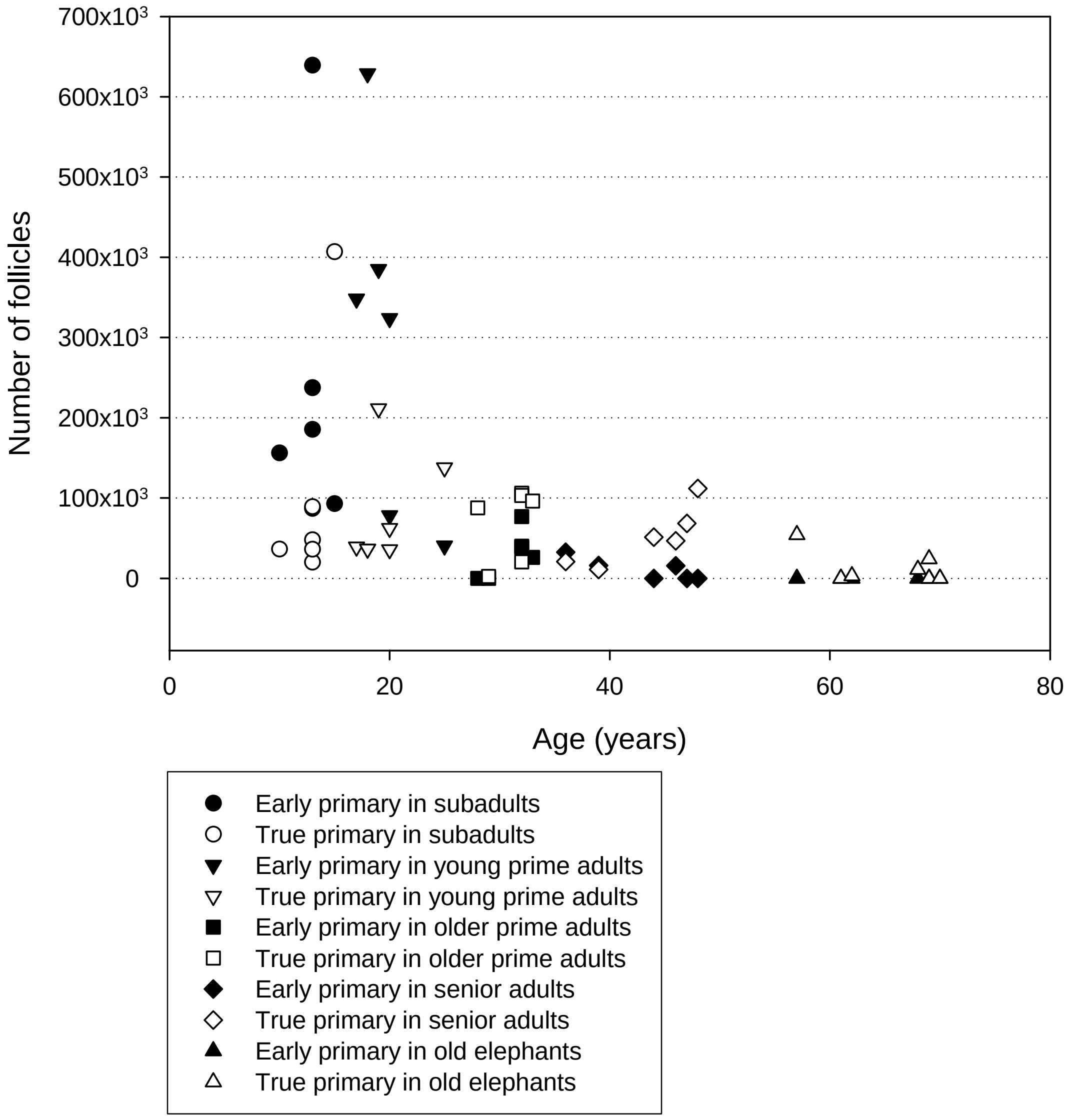

Figure 7.3 The numbers of early primary (EP)- and true primary (TP) follicles in the ovaries of African elephants of different ages

Table 7.3
True primary follicles (TP) as a percentage of total small follicles (SF) in the ovaries of elephants of different age groups

		Percentage		
Age group (years)	n	Median	25th Percentile	75th percentile
Subadults (10–15)	6	17.7	15.3	34.5
Young prime adults (16–25)	6	23.8	9.0	45.9
Older prime adults (26–35)	6	75.6	51.6	100
Senior adults (36–50)	6	87.5	40.5	100
Old (51–70)	5[a]	100	100	100

[a] Two of the 7 old elephant had no small follicles, and the percentage of all small follicles that were true primary did not apply to them

7.3.3. The relationship between reproductive status or tusklessness and the number of small follicles

The appearance of their ovaries suggested that the elephants belonged to one of four reproductive statuses: i) no antral follicle development (Figure 7.4a) and no CL but sometimes with CN, indicating post-partum anoestrus (Perry 1953); ii) few antral follicles (Figure 7.4b); iii) many antral follicles (Figure 7.4c) and iv) CL present (Figure 7.4d).

In the 10–15 year age group, 3 nulliparous females had a mean of 504 469 (SD 221 531) SF in their ovaries compared to a mean of 173 666 (SD 59 715) in the 3 females that were post calving ($P = 0.067$). Otherwise there was no relationship between either reproductive status ($P = 0.31$) or the tuskless state ($P = 0.46$) on follicle numbers.

7.3.4. Reproductive status of the old elephant

Table 7.2 shows that 6 of the 7 oldest animals in the study showed signs of recent ovarian activity in the form of antral follicles (Figure 7.4e), CL or large CN. A comparison of CN size in three 30–35-year-old cows is given in Table 7.4. The oldest animal, aged 70 years, had seemingly inactive ovaries with no SF reserve and no antral follicles present (Figure 7.4).

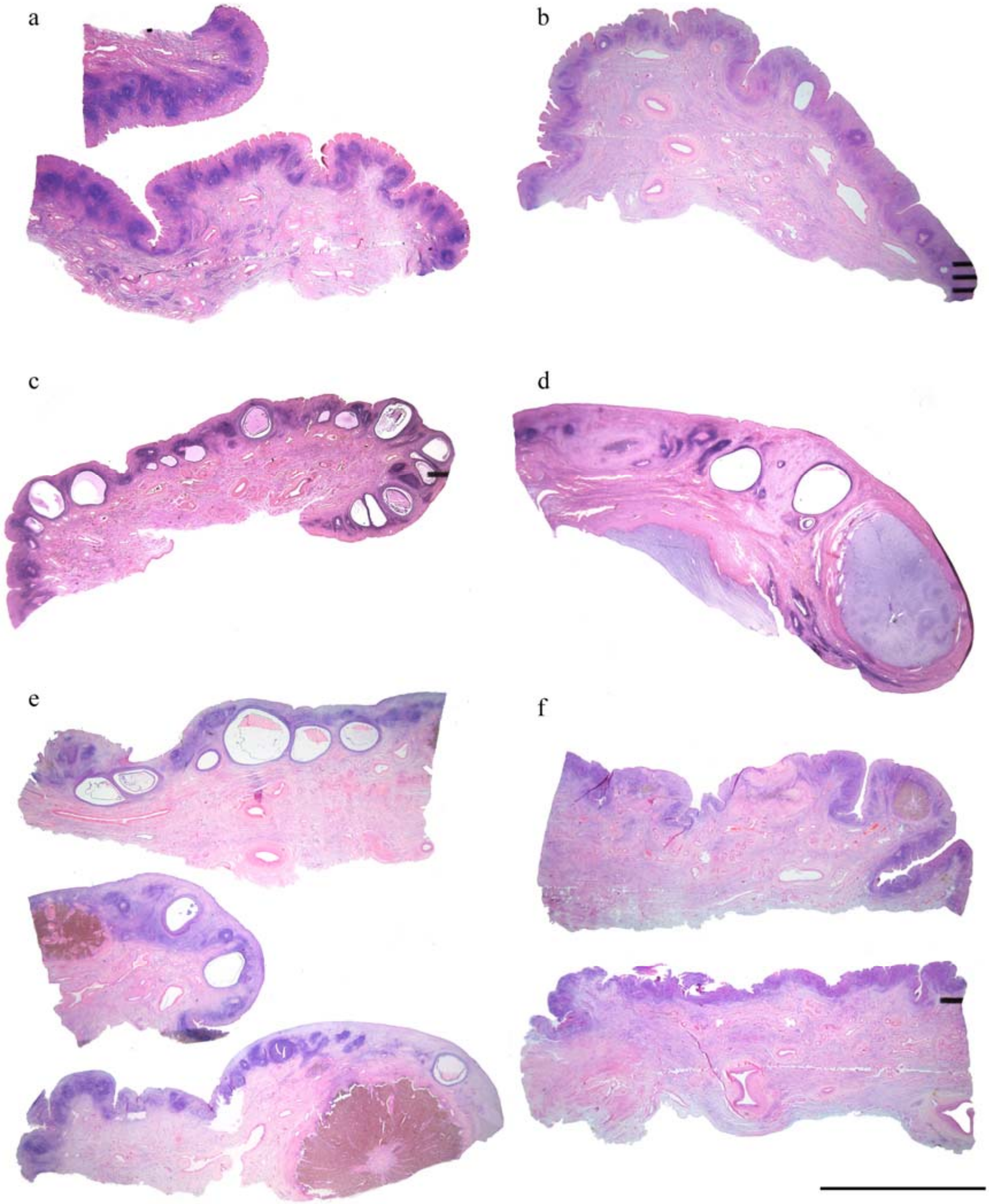

Figure 7.4 Photomicrographs of 25 µm sections cut in a transverse plain, perpendicularly to a longitudinal bisection of the ovary, revealing the cyclical changes within the cortex of the elephant ovary

a. An inactive-looking cortex from a cow in post-partum anoestrus.
b. Section showing a small degree of antral follicle development.
c. Section showing a large degree of antral follicle development.
d. Section showing antral follicles and a small corpus luteum.
e. Three segments from the 2 very active ovaries of a 69-year-old elephant.
f. Segments from the ovaries of a 70-year-old elephant which, apart from a small luteal remnant, are inactive. Scale bar = 10 mm.

Table 7.4
Comparative data of *corpora nigra* (CN) of known age in African elephants

			Corpora nigra	
Elephant ID	Age (y)	Status at death	Diameter (mm) [a]	Age (months) [b]
80	34	21 months pregnant [c,d]	5	67
11–17	30	5.7 months pregnant[c,d]	10	52
		Dam of 2.5 y old calf [e]		
11–12	32	Dam of 1.5 y old calf [e]	14	40

[a] The diameter of the largest CN in the elephant
[b] Estimated age of the cohort of CN that includes the largest one, assuming that the original CL formed at the beginning of the previous gestation and a 24-month lactation anoestrus preceded the current pregnancy
[c] Aged by fetal weight (Craig 1984)
[d] Assuming a 24-month lactation anoestrus preceded the current pregnancy
[e] Aged by calf jaw

7.4. Discussion

7.4.1. The follicle reserve after puberty

Natural variation in follicle numbers was found to be lower in the sexually mature age groups than during pre-pubertal life. The coefficient of variation for the numbers of SF in the two ovaries of an elephant was 11%, suggesting that the numbers of SF in the two ovaries of an elephant are quite similar.

This study shows that the size of the follicular reserve in African elephants depends on age. Comparing the follicular reserve of sub-adults (10–15 years of age), young prime adults (16–25 years of age), prime adults (26–35 years of age), senior adults (36–50 years of age) and old elephant (51–70 years of age) reveals that old elephant have lower follicular reserves than sub-adults and young prime adults, but similar to older prime adults and senior adults. When, led by the data, only the young prime adult elephant group and the older prime elephant group were compared, it was found that the former had significantly more SF than the latter. Although not statistically significant, the median number of SF also became numerically lower from older prime adults to senior adults and finally to old animals, but larger numbers of elephants per age group need to be examined to determine whether or not a significant trend underlies this numerical pattern. Assuming that follicular reserves of the elephants included in the current study are representative of those of African elephants in general, it follows that the follicular reserve shows a significant decline during the third decade of life and that it is not uncommon for old elephant to have either no follicles remaining in their ovaries or a small number, in the order of 10 000 or fewer.

The old elephants in the current study did not die of old age. Hence, the current study does not answer the question whether or not the ovaries of all African elephants eventually become depleted of their follicle reserve. The current study does, nevertheless, suggest that it is not uncommon for old elephants, in their 7th decade of life, to have no follicular reserve remaining and that depletion of the follicular reserve would constrain fertility of old African elephants. This study also suggests that although the follicle reserve may finally become exhausted in African elephants it does not do so until very late in the total lifespan, which is brought to a close by erosion of the last set of molar teeth. This is well beyond the 45 year age of average life expectancy of a female elephant noted in the long term study in Amboseli Game Park in Kenya by Moss and Lee (2011).

As a result of natural variation such sustained fertility will not exist in all aged animals. Some individual had unusually low numbers of SF. For example, one 29-year-old individual had a total of only 2400 oocytes remaining in her ovaries. In this study, this low level of reserve was otherwise only seen in animals older than 57 years of age.

7.4.2. The change in follicle numbers around puberty

In Chapter 6, using the same methods and animals from the same population as those of the current study, SF were counted in 8 elephants, aged 6–9 years, which are the years just before puberty. They had 1 261 593 (SD 676 417) SF, which was substantially and highly significantly more than the 339 091 (SD 232 134) of the 6 sub-adults, aged 10–15 years, which were examined in the current study (one-tailed t-test, P = 0.003). This comparison between studies suggest a sharp drop in the number of SF from around puberty until the age of 15 years, which is when sub-adults usually calve for the first time.

7.4.3. A switch in type of small follicle constituting the reserve

Irrespective of their age, all but two elephants had 150 000 or fewer TP. EP numbers were initially much higher than TP but became depleted by 45 years of age to leave only TP forming the follicle pool for the remainder of reproductive life.

When sub dividing the population of SF into EP and TP the current study showed that the numbers of TP in the ovaries of postpubertal elephants was below 200 000 (Figure 7.3). True primary follicles, expressed as a percentage of the small follicle pool, increased from below 2.6% in fetal life to levels reaching 7% in calves aged 0–3.4 years and levels reaching 16% in young animals aged 3.5–9.5 years (Chapter 6) The current study showed that, irrespective of their age, postpubertal elephants generally had 150 000 or fewer TP. In spite of their fairly constant number, the TP constituted a progressively larger proportion of all SF until the proportion reached 100% by about 45 years. This change in ratio while the number of TP remained fairly constant is due to a gradual reduction in the number of EP. It is not known whether the number of EP decreases mainly due to atresia or due to conversion to TP, or due to both processes. EP are no longer present from around the age of 45 and for the remaining reproductive years only TP type follicles hold the oocytes.

TP follicles have been classed as growing follicles or non-growing follicles in other mammals (Hansen *et al.* 2008; Picton 2001), the point of follicle activation being determined by the expansion of the first granulosa cells from a squamous to a cuboidal shape. As previously noted, elephant ovaries contain almost no TPM throughout life, their follicle pool or reserve being composed entirely of EP and TP. It is not known whether the SF designated as EP and TP in elephant are resting and non-growing, or whether they are growing very slowly. If the TP in elephant older than about 45 years are growing, they have to do so sufficiently slowly to ensure that the stock of TP present in the ovaries at 45 years of age may last a further 20–25 years.

The number of SF in the single nulliparous semi-domesticated elephant (age 28) included in the current study was within the usual range for her age, although all these follicles showed a TP morphology. Being nulliparous, the state of her follicle reserve may be compared to captive nulliparous zoo elephants that fail to breed beyond 30 years of age.

7.4.4. The value of the current study with respect to understanding infertility of Zoo elephants

The population of elephants maintained in zoos in North America and Europe is not self sustaining (Brown *et al*. 2004a; Dow *et al*. 2011; Proctor *et al*. 2010). In USA, 12% of adult females exhibit irregular oestrous cycles and 31% do not cycle at all.

It has been suggested one of the reasons for cessation of reproduction may be a direct result of ovarian ageing (Hermes 2000). That is, loss of the oocyte pool due to increased cyclicity in nulliparous zoo elephant. For example, a captive female elephant is likely to ovulate as many oocytes by the age of 12 years as a wild female does in her entire lifetime (Hermes 2000). Constant cyclicity is also known to lower the age of menopause in women (van Noord *et al.* 1997).

Data from the current study provides useful information to address this particular point. Only a tiny fraction of the original ovarian endowment of oocytes find their way to ovulation, with follicular atresia causing the demise of the bulk (Krysko *et al.* 2008). Such loss of oocytes takes place every day and continues throughout pregnancy and lactation in all mammalian species studied to date (Gougeon 2010). In women for example, according to a model of non-growing follicle numbers, at the peak of follicle activation at around 14 years of age a mean of around 880 (range 100–7 500) non-growing follicles per month enter the growth phase (Wallace & Kelsey 2010).

In the present study transitional and secondary follicles were observed in the ovaries of non-pregnant elephant at all ages except the very old animals in which few follicles still existed. Although these transitional and secondary follicles were not counted they appeared rare. Specimens from pregnant elephants in a previous study (Stansfield 2006) showed similar results. Although the presence of transitional and secondary follicles indicate early follicle growth from the reserve of EP and TP, further study is required to investigate their perceived rareness. In the light of this perceived rareness, the suggestion that more SF are activated from the reserve in nulliparous non-pregnant elephants is not necessarily correct, particularly because the early stages of initiation of follicle growth are thought to be independent of gonadotrophic stimulation (Berne *et al.* 2004). Small follicles are constantly being "activated" in a paracrine controlled environment, taking more than 120 days to pass from the primordial to the secondary stage in women. Growth to the pre-antral stage takes a further 85 days, at which point the follicles become gonadotrophin sensitive and may be recruited. Then follows the 8–12 day period of selection and dominance of one follicle some days prior to ovulation (Eshre 2005).

It would therefore be of interest to compare the number of oocytes within the ovaries of captive elephants with the cross sectional results reported here in wild elephants. To this end, the ovaries of the one semi-domesticated, nulliparous elephant aged 28 years (named MM) could be studied. The total number of oocytes in her ovaries (88 000) was within the acceptable range for her age but it was notable that she exhibited only TP follicles. Only one other wild elephant under 45 years of age had no reserve of EP follicles in her ovaries. She was aged 29 years and had only 2392 TP. Elephant MM had shown no oestrous activity throughout her life and was dominant in her "family group"over a 27-year-old bull and a 19-year-old cow which had similarly shown no breeding activity. At post mortem her ovaries contained a recent ovulation stigma, antral follicles up to 9 mm in diameter and some CL and small CN. In contrast, the wild 29-year-old mentioned above that showed the mentioned low ovarian reserve had inactive-looking ovaries with no antral follicle development at all. She did, however, exhibit some large CN from the pregnancy that produced the 2-year-old calf by her side. One further wild elephant was of interest in that she had an inverted number of TP:EP ratio (92 961 EP in relation to 406 967 TP) and was nulliparous at 15 years of age. She showed plenty of antral follicle development and some small CN from previous cyclical CL.

These isolated observations raise the question of whether prolonged nulliparity in the elephant may prematurely exhaust the EP pool in their ovaries. Although MM had a good supply of SF these were all at the TP stage and therefore equivalent to a wild elephant of around 45 years of age when some authors have reported a slowing in reproductive rate of wild elephants (Hanks 1972; Laws *et al*. 1970; Sherry 1975). Whether MM is representative of the follicle status of a mature nulliparous animal needs to be addressed by examining more ovaries from captive deceased animals when they become available at *post mortem* studies.

MM forms an important observation since although she was nulliparous she was in lean body condition at the time of death and had experienced a natural diet and a relatively stress free daily life, in marked contrast to the normal experience of zoo elephants. However, her social status was not normal as the 3 companion animals had been captured as calves during culling operations and had been hand-reared together throughout their lives. It is likely that her familiarity with her companions prevented her overt expression of oestrus although it did not suppress ovarian cyclicity (Freeman 2004).

7.4.5. The relationship between tusklessness and the follicular reserve

In African elephant inherited bilateral tusklessness is sex-linked in females (Steenkamp *et al.* 2007) and increasing numbers of tuskless animals within Zimbabwe is thought to be a result of selective ivory poaching. Apart from the current study, which showed no effect, no report has been found on the effect of tusklessness on ovarian function.

7.4.6. Reproductive senescence

Turning to the oldest samples collected, 7 sets of ovaries from elephants aged 57–70 years were available for this study (Table 7.2). Low numbers of SF were counted in the ovaries of all these animals thereby causing the coefficient of error to be above normally acceptable levels when using the same stereological protocol as for younger animals. Due to this very low number of SF, and some cases in which no follicles at all were counted, it was decided it would be fruitless to cut and examine any greater number of sections (Charleston *et al.* 2007). When comparing the three 57–62-year-old individuals with the four 68–70-year-old individuals, a higher number of SF was counted in the younger animals. Nevertheless, the four oldest animals still had from zero to 24 278 (median 7067) SF, which is appreciably higher than the average of only 1000 oocytes remaining in the ovaries of women when they approach menopause at around 51 years of age (Faddy & Gosden 1996).

The structures left behind from the large CL of pregnancy become increasingly dark brown in colour and are termed *corpora nigra*. They have been suggested to persist for at least 77 months from the time of CL formation (Smith & Buss 1975) and therefore could be used as a guide to the interval since last calving. The size of the CL of pregnancy vary greatly as will the CN that persist from them (Lueders *et al*. 2011; Stansfield & Allen 2012). However, data from CN of known age presented in Table 7.4 suggests that not many years had past since 6 of the 7 oldest females studied had produced large CL of pregnancy.

The present finding of sustained reproductive capacity into old age is supported by behavioural studies on elephants in Amboseli National Park in Kenya where age-specific fecundity has been recorded in a population of elephants studied closely since 1972 (Moss 2001; Moss & Lee 2011). This has shown that, of 38 females in the study group with well known histories that had reached 50 years of age, most continued to reproduce and only 9 appeared to have stopped breeding, with 7 years having past since they had last given birth. Twelve females in the group over 60 years of age had given birth, with the most recent calving intervals averaging 4.75 years compared to 4.5 in the general population. This difference of 0.25 years represents less than the time period of one oestrous cycle thereby indicating a remarkably constant level of fertility over a period of some 50 years. This contrasts with women, where fertility rates begin to fall from the early 20's. Although there may be numerous causes of the difference in maintenance of fertility rates between elephant and women and the answer is not known, one may be that a different regulation of follicle reserve takes place in the elephant compared to women.

This remarkable reproductive longevity of African elephants has been observed and documented across Africa including South Africa (Freeman *et al.* 2008), Uganda (Laws 1969; Perry 1953), Zambia (Hanks 1972), and Zimbabwe (Sherry 1975; Williamson 1976). The only other long-lived mammals capable of supplying oocytes right up to death at age of maximum life expectancy are the baleen whales which may live and breed till over 100 years of age (Mizroch 1981).

7.5. Conclusion

In conclusion, this study shows that the size of the follicle reserve in the African elephant depends on age with a noticeable decline in the number of SF after the age of 25. Despite the trend for a depletion in SF throughout life, some old elephant still had low reserves of

SF in their ovaries at the time of culling. The EP follicles that constituted the follicle reserve in younger animals were no longer visible in the ovaries of elephant after the age of 45, and TP follicles only formed the reserve for their remaining years of life.

The content of this chapter will be submitted in a slightly different format to an accredited journal.