

Observations on ovarian structure and development of the southern giraffe, *Giraffa camelopardalis giraffa*

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The occurrence of vesicular follicles and corpora lutea (CL), without evidence of ovulation, in late fetuses and during prepubertal life was confirmed in the southern giraffe. In this, and other respects, the pattern of ovarian development is similar in the southern giraffe to the northern subspecies. This early ovarian activity recedes during late prepubertal life. Numerous accessory CL were present in pregnant and non-pregnant giraffe but they did not attain the size of the true CL of pregnancy. The latter undergo an initial phase of rapid enlargement lasting about 100 days, followed by a period of recession till mid-pregnancy and a second growth phase which persists until term (457 days). The CL of pregnancy rapidly regresses after parturition. The accessory CL regress slowly and form normal looking corpora albicantia (CA). These CA are most abundant during early adult life as also are vesicular follicles.

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Die voorkoms van vesikulêre follikels en corpora lutea (CL), sonder die bewys van ovulasie, in gevorderde fetusse en gedurende die pre-puberteitleeftyd, is bevestig in die suidelike kameelperd. Vir hierdie, en in ander opsigte ook, is die patroon van eierstokontwikkeling in die suidelike kameelperd dieselfde as vir die noordelike subspecies. Hierdie vroeë aktiwiteite in die eierstok verminder gedurende die laat puberteitperiode. Verskeie bykomstige CL was teenwoordig in dragtige en nie-dragtige kameelperde maar dit het nie die grootte bereik van die ware CL tydens dragtigheid nie. Laasgenoemde ondergaan 'n aanvangsfase van vinnige groei wat ongeveer 100 dae duur, opgevolg deur 'n periode van agteruitgang tot middel-dragtigheid en 'n tweede groeifase wat volhou tot die einde (457 dae). Die CL van dragtigheid verminder vinnig na die dier gebaar het. Die bykomstige CL gaan stadig agteruit en vorm normaal geskape corpora albicantia (CA). Hierdie CA asook die vesikulêre follikels is die volopste gedurende die volwasse tydperk.

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Reports by Kellas, van Lennep and Amoroso (1958), Kayanja and Blankenship (1973) and Gombe and Kayanja (1974) refer to an unusual form of follicular development, and the production of apparently normal corpus luteum-like structures in late fetal and prepubertal ovaries of the two northern subspecies of giraffe *Giraffa c. camelopardalis* Linn. and *G. c. tippelskirchi*. We have studied ovaries of the southern subspecies *G. c. giraffa* Boddaert, and from one individual of the subspecies *G. c. angolensis* and compared gross and micro-anatomy of these with the published accounts. We have found that although the overall picture of ovarian activity in the various subspecies of giraffe is similar, some differences do exist.

Material and Methods

Ovaries were collected from 34 giraffes, varying in age from two weeks to 20 years, culled in the eastern Transvaal, South Africa. Four fetuses were also collected and the ovaries of one giraffe from South West Africa (*G. c. angolensis*) were also studied. Ages were determined following Skinner and Hall-Martin (1975) for fetuses, and Hall-Martin (1976) postnatally.

The ovaries were fixed (10% formalin or Bouin's fluid), weighed, measured and embedded in paraffin wax. Serial sections (5 μ m) were stained in Delafield's haematoxylin and eosin or in Martius-Scarlet-Blue (Disbrey & Rack 1970). Diameters of antral follicles, corpora lutea (CL) and corpora albicantia (CA) were measured following Mossman and Duke (1973) for prepubertal giraffes and Rowlands and Heap (1966) for pubertal giraffes. Measurements are given as the mean \pm S.E.M.

Observations

The prenatal ovary

The two younger fetuses were 273 and 274 days old, the mean dimensions of the ovaries were 1,2 \times 1,4 \times 0,5 cm and mean mass was 1,0 g. The two older fetuses were 405 and 414 days old, of a 457 day gestation (Skinner & Hall-Martin 1975), and have ovarian dimensions of 2,1 \times 1,5 \times 1,3 cm and a mean ovary mass of 2,8 g.

Large numbers of naked oocytes were present in the superficial layer of the cortex of the younger ovaries, while in the older ones many had degenerated. The innermost zone of the cortex contained stromal tissue and some secondary follicles. The medulla of all fetal ovaries

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was crowded with primary, secondary and vesicular follicles, most of which showed signs of degeneration. The largest healthy vesicular follicles had volumes of 129–206 mm³; the membrana granulosa was attenuated and some cells of the theca interna gave evidence of secretory activity. Several haemorrhagic follicles, 14 to 22 mm³ in size, were found in the younger ovaries, as also well developed rete ovarii tubules 40–80 µm in diameter.

Well-vascularized CL of the type reported by Kellas *et al* (1958) and Kayanja and Blankenship (1973) were found in the medulla of the fetal ovaries. Four CL were present in one ovary of one of the earlier fetuses but each ovary of both of the older fetuses contained four to nine CL. The largest CL had a volume of 20,6 mm³ but many were compressed or flattened. The individual lutein cells measured 12 to 20 µm in diameter and their nuclei between 4,1 to 5,7 µm in diameter. The very conspicuous vascular network throughout the fetal ovaries was indicative of intense activity in the CL and of follicular stimulation.

The mode of formation of these CL is not entirely understood but we identified the 'discrete spherical masses of granulosa-like cells surrounding an oocyte' as described by Kellas *et al* (1958) as follicles (usually secondary). Some of the CL contained material which stained like follicular liquor and they were therefore identified as originating from vesicular follicles. These structures were penetrated by large sinusoidal capillary vessels and contained a conspicuous network of reticular fibres. They were most likely formed by the rupture of the membrana propria and the invasion of the follicle by granulosa cells which became luteinized; this was followed by the degeneration of the oocyte. In other follicles the capillary vessels burst and a haemorrhagic follicle was formed, the cellular debris being phagocytosed. Because the luteal tissue of the fetal CL is sometimes continuous with the theca interna of what was a vesicular follicle, as also noted by Kayanja and Blankenship (1973), it is possible that thecal cells also play a role in the development of the fetal CL.

The prepubertal ovary

The mean mass of the ovaries of a two-week-old calf was only 2,4 g, but this increased to 8,0 g for those of a four-year-old giraffe, the dimensions being 3,3 × 2,2 × 1,6 cm. As the ovaries developed the coelomic epithelium became increasingly attenuated and the numbers of oocytes and primordial follicles declined. Secondary and vesicular follicles (mostly atretic) with wide thecae interna were present. Thecal type interstitial gland tissue and rete ovarii tubules were conspicuous. Up to 16 CL per ovary, as large as 381,3 mm³, were found. These CL were histologically similar to those of adults; their luteal cells measured 26–33 µm with nuclei 10,1–11,9 µm in diameter. In the older immature individuals the luteal cells were regressing, and CA were identified for the first time in a four-year-old giraffe.

The mature ovary

In one six-year-old non-parous female the ovaries were still small (3,6 × 2,2 × 2,0 cm, mean mass 10,4 g) and the multiple CL of the immature period were recognizable as CA surrounded by a broad band of predominant-

ly collagenous material. The size of adult non-pregnant ovaries was about 4,9 × 3,7 × 2,2 cm with a mean mass of 46,1 ± 3,0 g and ovaries of pregnant adults were 5,4 × 4,0 × 2,7 cm with a mean mass of 75,5 ± 4,3 g.

The main histological features of giraffe ovaries are similar to those of other mammals especially the ruminants (Kayanja & Blankenship 1973, Mossman & Duke 1973, Hall-Martin 1975).

The follicles

The number of vesicular follicles increased during early adult life and then declined steadily after the age of 10 years to a minimum of 24 found in a 20-year-old individual (Fig. 1). There were more follicles in the ovaries of pregnant than non-pregnant giraffe (134,3 ± 26,7 *cf* 108,1 ± 29,5) and more were present during late than early gestation, but these differences were not statistically significant. As gestation (*x*) advanced the volume (*y*) of the largest follicle decreased (Fig. 2) as described by the equation $y = -1,757x + 133,4$ ($r = -0,761$; $P < 0,001$) indicating persistence of follicles through gestation, even though they are declining in size. The size of the largest follicle in non-pregnant females (Fig. 2) indicates a post-partum follicular stimulation probably related to the return to oestrus.

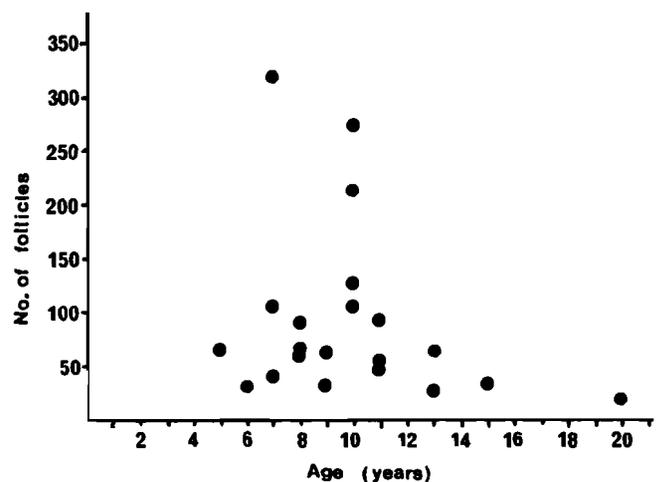


Fig. 1 The number of vesicular follicles in relation to age of giraffe.

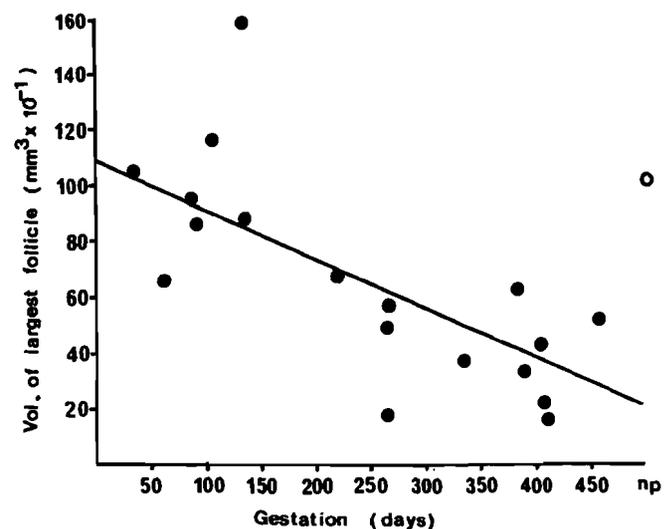


Fig. 2 Volume of largest vesicular follicle in relation to gestation age in giraffe; o = mean volume ($n = 7$) of largest follicle in non-pregnant (np) females.

The corpus luteum

The largest CL in non-pregnant giraffe were smaller than those in pregnant individuals ($4\ 140 \pm 1\ 130\ \text{mm}^3$ cf $26\ 431 \pm 3\ 620\ \text{mm}^3$; $P < 0,001$). The number of CL present declined with advancing age. Corpora haemorrhagica were not found but accessory CL (developed by luteinization of unruptured follicles) were common. Secondary CL, produced from ovulation during pregnancy, were not found.

CL volume increased rapidly for about 100 days following ovulation, the lumen being completely occluded by 30 days post-conception. This initial phase was followed by a steady decrease in CL size to about 250 days of pregnancy and thereafter the trend was again reversed (Fig. 3). The changes in CL volume (Fig. 3a) and ovary mass (Fig. 3b) are clearly not linear. Polynomial regressions were therefore used to plot these curves. Regression of the CL occurred rapidly after parturition.

Accessory corpora lutea

When fully developed these CL had a volume of about $65\ \text{mm}^3$ and were invariably smaller than the 'true' CL resulting from ovulation. They were more frequent in young females (Fig. 4) and occurred as frequently in non-pregnant as in pregnant giraffes. The number of accessory CL decreased with age, except for one 13-year-old pregnant female (three days pre-partum) in which there were 24. The presence of CA in the four-year-old subadult and five to six-year-old non-parous adult; and the evidence that the last of the CL of the immature period were regressing in these individuals, suggests that the formation of accessory CL occurs only after the first conception. Their occurrence as frequently in non-pregnant as in pregnant individuals suggests that they last longer than the CL of pregnancy, or possibly that some are also formed in non-pregnant parous females.

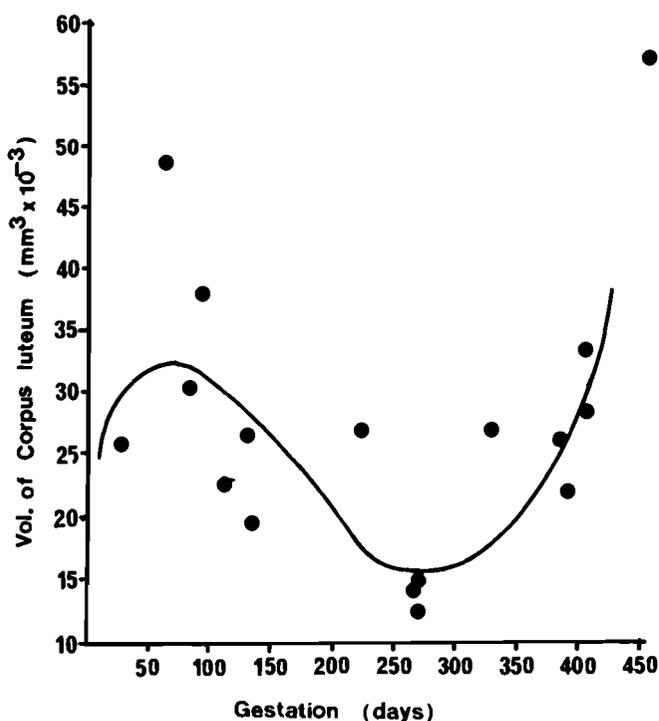


Fig. 3(a) Volume of corpus luteum in relation to gestation age in giraffe.

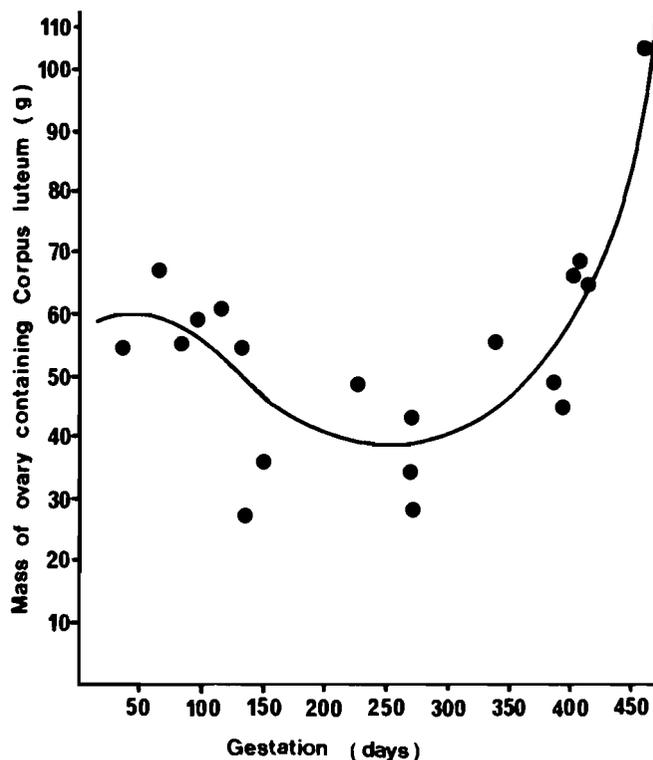


Fig. 3(b) Mass of ovary containing the CL in relation to gestation age in giraffe.

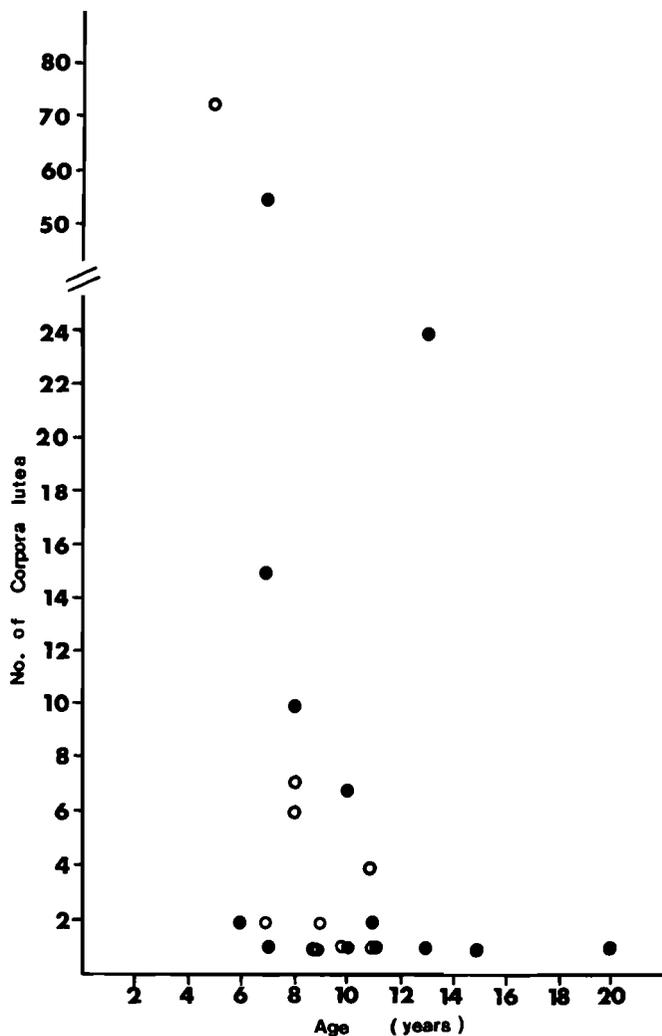


Fig. 4 Number of corpora lutea and accessory corpora lutea combined as a function of age in adult female giraffe; o = non-pregnant, ● = pregnant.

The corpus albicans

These were present in the ovaries of all post-pubertal individuals except for one six-year-old primiparous giraffe whose ovaries contained 55 CL. The greatest number of CA (24) were found in another six-year-old female and presumably were due to persistence of regressing CL of the pre-pubertal period. Except for this giraffe, the number of CA increased with age (Fig. 5a), but not significantly. There was no significant difference in numbers of CA found in pregnant and non-pregnant individuals. The decrease in mean volume of the CA during pregnancy is curvilinear (Fig. 5b) where $r = 0,75$; $P < 0,001$.

Alternation of ovarian function

Both ovaries were equally active, ovulation alternating between them. Of 28 pairs of adult ovaries examined the largest CL occurred 13 times in the left ovary, and 15 times in the right ovary; there were 76 CL and 74 CA in the left ovaries and 75 CL and 83 CA in the right ovaries respectively. The largest vesicular follicle occurred in the opposite ovary from the one containing the largest CL in 26 of the 28 pairs ($P < 0,001$) and the largest CA occurred contralaterally to that containing the largest CL in 21 of the 28 giraffes ($P < 0,025$).

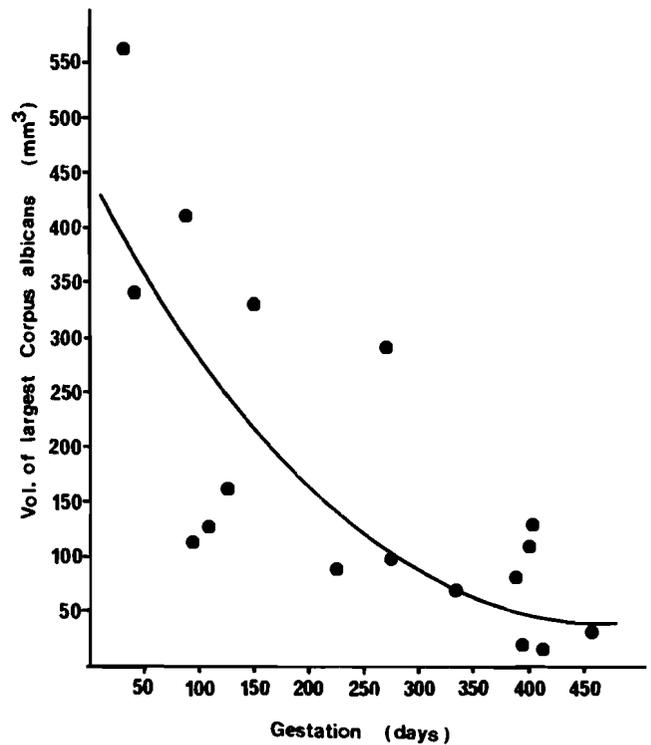


Fig. 5(b) Volume of largest corpus albicans at gestation age.

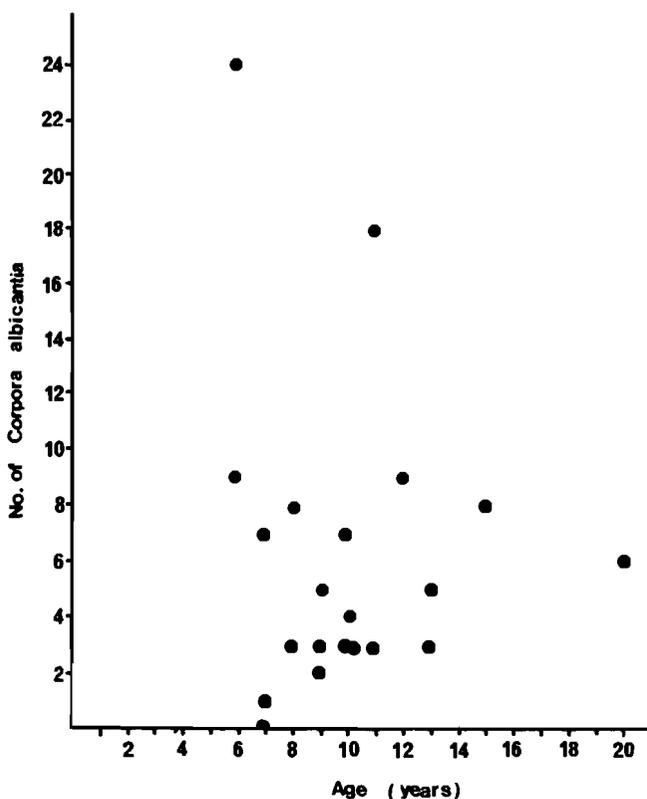


Fig. 5(a) Number of corpora albicantia in relation to age of female giraffe.

Discussion

It is apparent that the type of gonadal stimulation which occurs in the fetus of the giraffe is completely different from the hormones stimulating the massive development of interstitial cells observed in fetal equids (Rowlands 1964; Smuts 1976) and phocids (Amoroso, Harrison, Harrison Matthews & Rowlands 1951). The time of onset of these changes in both types of gonadal stimulation is similar, but whereas the condition recedes pre-natally in the horse and seal, follicular development and luteinization in the giraffe ovary persist for several years during pre-pubertal life.

Although the overall picture of ovarian activity in the different subspecies of giraffe is similar, there are some differences. For example, the ovaries of the Masai giraffe 'shortly before birth', according to Kayanja and Blankenship (1973) are much smaller than those of the largest fetus of our series of *G. c. giraffa* (1,6 cm³ and 1,6 g cf 4,1 cm³ and 2,8 g). Such differences could be attributed to many factors — individual variation, fetal age, number of luteal bodies present or to sub-specific variation. The body mass of 52 kg which they give for a fetus 'shortly before birth' is only half that of a full term fetus from our area (Skinner & Hall-Martin 1975).

We agree with Kayanja and Blankenship (1973) that the CL in the ovaries of fetal and juvenile giraffes contain adult type luteal cells but that they are somewhat smaller. We did not determine whether these cells were able to synthesize steroids, as did Gombe and Kayanja (1974) but such activity might be adduced from the histological condition of the fetal uterus.

Mossman and Duke (1973) suggested that these bodies are not true luteal glands but thecal-type interstitial

bodies (corpora atretica) derived from medullary cords. We did not identify medullary cords in the present study nor were they mentioned by Kellas *et al* (1958).

The large numbers of developing follicles found in the early fetal ovaries and their relative sparseness in those closer to term indicate that there is at least one wave of follicular growth and proliferation followed by a cessation of this activity in fetal life. This situation is common among mammals (see Rowlands & Weir, 1977).

The data also suggest that CL regression takes place slowly throughout prepubertal life and that in most cases at puberty only CA remain. We found no evidence to support Gombe and Kayanja's (1974) statement that these CL are maintained cyclically until puberty is reached.

The presence of multiple CL in pregnant adults in our material is important as Kayanja and Blankenship (1973) noted that accessory CL were not formed during pregnancy in the giraffes which they examined. Our specimen of *G. c. angolensis* was 13 years old and in common with three out of four *G. c. giraffa* females of similar age or older she had no accessory CL. The *G. c. angolensis* material did not differ in any other respect.

The change in size of the CL of pregnancy is unusual but not unknown among mammals e.g. the laboratory rat (Perry, 1971) and wildebeest *Connochaetes taurinus* (Watson, 1969). Kayanja and Blankenship (1973) observed that the CL of pregnancy in their material were largest during the first third of pregnancy and decreased 'towards the end of gestation'. As they did not measure these CL it is difficult to compare their material directly with ours. It is possible, however, that most of their ovaries came from females in early gestation and that they were probably observing the decline in CL size from about day 100 to day 250 gestation as shown above.

In the rat, the renewed activity of the CL in late pregnancy is attributed to the secretion of a placental luteotrophin. The existence in the giraffe of some change in the endocrine milieu during mid-pregnancy is also probable. This view is supported by the biochemical observations of Gombe and Kayanja (1974), particularly the rising levels of luteal progesterone in the second half of pregnancy, and by the much earlier unconfirmed report of Wilkinson and de Fremery (1940), of the presence in the urine of the pregnant giraffe of a gonadotrophin similar to that excreted by pregnant woman (HCG). This hormone was not detected on day 243 but positive indications of its presence were first obtained on day 322 and 337.

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