Ovarian Cycle of *Crotaphytus collaris* (Reptilia, Lacertilia, Iguanidae) from Arkansas with Emphasis on Corpora Albicantia, Follicular Atresia, and Reproductive Potential

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**ABSTRACT**—Macroscopic and histological analyses of the ovarian cycle of *Crotaphytus collaris* were conducted during 1971 and 1972. The reproductive season extends from April to late June; examination of ovarian histosections revealed two distinct sets of corpora lutea, indicating that two clutches of eggs are produced per season. The average clutch size was 6.4. Corpora lutea regress into permanent ovarian scars (corpora albicantia). Consequently, corpora albicantia may be used to estimate total reproductive potential for an animal. In addition, two types of atretic follicles were staged according to their degenerate morphology. The similarity and persistence of these ovarian structures (atretic follicles and corpora albicantia) may limit the value of macroscopic interpretations.

**INTRODUCTION**

Cagle (1953) suggested that counts of corpora albicantia (remnants of regressed corpora lutea) might be helpful in determining the number of eggs ovulated by reptiles following a breeding season. References to these ovarian structures have appeared infrequently in the literature (Ballinger, et al., 1972; Cagle, 1953; Ernst, 1971; Goldberg, 1970, 1973; Telford, 1969). Telford (1969) determined that counts of corpora albicantia constitute a useful alternative method for estimating the reproductive potential and population structure in the Japanese lacertid lizard, *Takydromus tachydromoides*.

A total of 120 adult female collared lizards was collected by sampling weekly during the 1971-1972 activity seasons. The animals were noosed or taken by hand from cedar glades or abandoned rock quarries along the White River drainage from Izard County northward in Arkansas. Elevation ranged from 112 to 355 m. The lizards were killed by injecting sodium pentobarbital and fixed in 10% formalin. Standard histological techniques were used to prepare 78 right ovaries for light microscopy. Ovaries were embedded in paraffin, sectioned into 10 μm serial ribbons, and stained with Harris’ hematoxylin and eosin. Several left ovaries were also sectioned. Ovaries with yolking follicles were not sectioned. In addition, counts and measurements of lengths of immature ovarian follicles (<3 mm), yolked ovarian follicles (3-13 mm), oviducal eggs, and corpora lutea were made from the left ovaries of 96 individuals. All measurements were performed with the use of a vernier caliper or ocular micrometer to the nearest 0.1 units. Prepared slides and specimens are currently in the possession of the author.

RESULTS AND DISCUSSION

Ovarian Cycle.—The reproductive cycle of Crotaphytus collaris females in Arkansas conforms to the criteria proposed by Tinkle, et al. (1970) in that this species matures early and has large, multiple-brooded clutches. The ovarian cycle is similar to the Callisaurus-Holbrookia complex described by Pianka and Parker (1972) in these ways: (1) age at first breeding is one year or less; (2) clutches are large and multiple; (3) average clutch size is 6.4, ranging from 3 to 11 (clutch size in Callisaurus and Holbrookia is ca. 5.2, range 2-12) and (4) reproduction peaks in late spring and/or summer. Female collared lizards reach sexual maturity in 4-5 months, excluding hibernation (Fitch, 1956) and possess yolked ovarian follicles at a snout-vent length (SVL) of 78 mm. Ferguson (1976) clarified the reproductive cycle of breeding females by dividing the cycle into eight stages based on postnuptial coloration, fat body weight, and ovarian condition. In Arkansas, vitellogenesis begins in the follicles of C. collaris one to two weeks after emergence from hibernation in early spring and continues for approximately one month until ovulation in May or June. Oviposition occurs one to two weeks after ovulation. Egg incubation requires about 72 days, and hatchlings appear from mid-July through September.

Immature ovarian follicles number from 13-24 per ovary in adult females from early April and are translucent to creamy white. Enlarging follicles (>3 mm in diameter) become yellowish-orange as yolking begins. One to six follicles per ovary rapidly increase in size until ovulation at 12-13 mm in diameter. Older females usually possess yolked follicles before first-year animals, and females that develop a second clutch begin follicular enlargement immediately following ovulation of the first clutch. The monthly distribution and size of ovarian follicles are shown in Table 1.

In 1971 and 1972, ovigerous females (N = 11; 17) were collected from 15 May until 28 June and 11 May until 30 June, respectively. Oviducal eggs varied from 15.1 to 20.5 mm in length and from 8.6 to 12.4 mm in width. Right oviducts contained a total of 89 eggs, and left oviducts, 79 eggs (difference not significant at 5% level).

Contralateral ovum displacement may be inferred when the number of oviducal eggs differs from the number of corpora lutea in the corresponding ovary (Cuellar, 1970). In the 28 gravid females autopsied in which counts of oviducal eggs and corpora lutea were made, ova had been discharged into the contralateral oviduct in 8 animals (28%). The transfer of only one ovum was recorded. In 5 females the ovum moved from the left ovary to right oviduct, and in 3, the movement was from right to left. Hipp (1977) found that the oviducts of 19% of the gravid collared lizard females he examined contained displaced ova.

Postovulatory follicles, or corpora lutea, are found in ovaries from mid-May until early
TABLE 1. Monthly distribution of ovarian follicles from the left ovaries of *Crotaphythus collaris* females from Arkansas. Numbers in parentheses represent percentage values.

<table>
<thead>
<tr>
<th>Sampling Period</th>
<th>N</th>
<th>SVL Range</th>
<th>0-1 mm</th>
<th>1-2 mm</th>
<th>2-3 mm</th>
<th>&gt;3 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Apr-14 Apr</td>
<td>9</td>
<td>73-93</td>
<td>43(30)</td>
<td>52(37)</td>
<td>39(27)</td>
<td>8(6)</td>
</tr>
<tr>
<td>15 Apr-10 May</td>
<td>14</td>
<td>71-98</td>
<td>80(24)</td>
<td>68(21)</td>
<td>55(17)</td>
<td>126(38)</td>
</tr>
<tr>
<td>11 May-31 May</td>
<td>19</td>
<td>85-103</td>
<td>105(36)</td>
<td>86(29)</td>
<td>69(23)</td>
<td>33(12)</td>
</tr>
<tr>
<td>1 Jun-30 Jun</td>
<td>25</td>
<td>78-101</td>
<td>153(44)</td>
<td>128(37)</td>
<td>47(14)</td>
<td>17(5)</td>
</tr>
<tr>
<td>1 Jul-31 Jul</td>
<td>19</td>
<td>82-97</td>
<td>133(48)</td>
<td>113(41)</td>
<td>29(10)</td>
<td>4(1)</td>
</tr>
<tr>
<td>1 Aug-31 Aug</td>
<td>8</td>
<td>77-96</td>
<td>88(48)</td>
<td>42(34)</td>
<td>22(18)</td>
<td>0</td>
</tr>
<tr>
<td>1 Sep-16 Sep</td>
<td>2</td>
<td>80-88</td>
<td>11(49)</td>
<td>8(35)</td>
<td>4(17)</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td>96</td>
<td>583</td>
<td>497</td>
<td>265</td>
<td>188</td>
<td></td>
</tr>
</tbody>
</table>

August. Regression of corpora lutea is shown in Figure 1. The recently ruptured follicle measures about 6 mm in diameter and appears as a bright red oval structure with a white matrix (luteal cells) plugging the cicatrix. The rapid shrinkage of the corpus luteum is probably due to a decrease in vascularization of the theca. A gradual decrease in size from 4 mm to around 1 mm occurs from the second to the fifth week following ovulation. During this time the corpus luteum appears yellowish-orange in color and elliptical in shape.

A predominant intraspecific feature of reproductive cycling in many North American lizards is seen in the seasonal and geographic variation in clutch size and frequency (Goldberg, 1973, 1975; McCoy and Hoddenbach, 1966; Tinkle, 1969; Tinkle and Ballinger, 1972; Vitt, 1977). Fitch (1956) observed a female *C. collaris* in northeastern Kansas that laid two clutches in one season, but Ferguson (1976) noted the unlikeliness of this occurring in northcentral Kansas. The production of multiple (second) clutches was determined in the present study by the following criteria: (1) concurrent yolked ovarian follicles and oviducal eggs, (2) concurrent yolked ovarian follicles and corpus lutea, and (3) two distinct sets of corpus lutea. Counts of yolked ovarian follicles and oviducal eggs, and those of yolked ovarian follicles and corpus lutea were determined macroscopically, while histological examination aided in counting sets of corpora lutea (Table 2). In Texas, Hipp (1977) observed two peak periods of vitellogenesis in April and June in older collared lizards, but stated that only 2 of 35 females showed direct evidence of multiple clutches. Field recapture data, showing two distinct periods of weight loss for a female during the reproductive season, can also be used to indicate oviposition of more than one clutch (Fitch, 1956; Trauth, 1974).
Corpora Albicantia.—Corpora lutea do not completely degenerate in *C. collaris* ovaries, but remain as minute (≈ 0.3 mm), darkly pigmented structures called corpora albicantia (Fig. 2A). As stated earlier, corpora albicantia are uncommon in reptiles. None were identified in *Cophosaurus texanus* (Ballinger, et al., 1972). Similarly, Goldberg (1973) found complete degeneration in *Sceloporus occidentalis*. Ernst (1971) was able to count corpora albicantia in the turtle, *Chrysemys picta*, but noted their disappearance when a new clutch of eggs was ovulated. Other than these in *Crotaphytus collaris*, I have been able to identify corpora albicantia only in the glass lizards, *Ophisaurus attenuatus* and *O. ventralis* (Trauth, unpublished data).

As a rule, the presence of pigmented cells surrounded by a dense layer of connective tissue was used to distinguish a corpus albicans from a regressed corpus luteum in collared lizard ovaries. These cells may be macrophages since they contain phagocytized particulate matter. However, this criterion was useful only after the corpus luteum had regressed to at least 0.3 mm in diameter, at which time they were referred to as corpora albicantia. Eventually the outer layer of connective tissue disappears leaving only a circular, blackish-orange mass of cells (Fig. 3). Corpora albicantia resemble the solid, darkly pigmented and densely packed masses of cells that are found inside degenerate atretic follicles. The significance of this similarity will be discussed later.

Follicular Atresia.—Two types of follicular atresia were identified in *C. collaris*. One involved the degeneration of immature follicles, and the other involved degeneration of yolked ovarian follicles or accessory follicles (Hoddenbach, 1966) which failed to ovulate. Atresia of immature follicles occurs throughout the activity season and evidently during hibernation, since ovaries of females in early spring possess atretic follicles in various stages of regression. Atretic accessory follicles (aaf) appear during the period of yolk deposition from April through June. The morphology of both types is different in respect to size, persistence of a central yolk mass (nucleoplasm?), and the degree to which the follicular granulosa cells participate in resorption of the ooplasm. The descriptive morphology and staging of atretic follicles are summarized in Table 3.

Although degeneration of immature follicles can best be seen by microscopic
examination, macroscopically, they possess a white central yolk mass surrounded by a clear translucent border. They rarely exceed a diameter of 2.0 mm and are most numerous during July. Older females (> 93 mm SVL) tend to possess more atretic follicles than younger females, with as many as 28 observed in various stages in a single ovary. The time for complete resorption is uncertain, since those that possess pigmented masses of cells (Fig. 3A-C) may persist for life in an animal. Atresia of this type has recently been described in Sceloporus jarrovi and S. occidentalis (Goldberg, 1970, 1973) and in Agama agama (Eyeson, 1971). Initial degeneration (Stage 1) of the oocyte involves the disappearance of the zona pellucida, hypertrophy of the theca, and the detachment of the follicular granulosa. The ooplasm becomes acidophilic, vacuolated and somewhat disorganized. The granulosa cells are seen invading and phagocytosing the yolk (Fig. 4A).

As regression proceeds the size of the follicle is greatly reduced, ranging from about 0.3-0.9 mm. Stage 2 atretic immature follicles (aif) are most evident during July and reflect the cessation of breeding activity. This stage (2) is characterized by a thin and irregular theca. A central yolk mass is persistent and may contain phagocytic leukocytes. Fibroblasts and connective tissue may fill the cavity of the follicle vacated by the absorbed ooplasm.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Atretic Condition</th>
<th>Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Early Regressing Immature Follicle. Theca appears hyperemic; the zona pellucida disappears. A detached granulosa layer invades the acidophilic ooplasm (yolk).</td>
<td>Early Regressing Accessory Follicle. Ooplasm is highly flocculent and vacuolated. Thca has hypertrophied; phagocytic cells are present throughout the yolk.</td>
</tr>
<tr>
<td>1'</td>
<td>Early Regressing Accessory Follicle. Ooplasm is highly flocculent and vacuolated. Thca has hypertrophied; phagocytic cells are present throughout the yolk.</td>
<td>4868-1496</td>
</tr>
<tr>
<td>2</td>
<td>Late Regressing Immature Follicle. Follicle is flaccid and irregular. Thca is reduced; few granulosa cells are present. A central yolk mass is persistent.</td>
<td>Late Regressing Accessory Follicle. Thca is hyperemic. A festooned follicular epithelium exhibits giant cells which ingest yolk.</td>
</tr>
<tr>
<td>2'</td>
<td>Late Regressing Accessory Follicle. Thca is hyperemic. A festooned follicular epithelium exhibits giant cells which ingest yolk.</td>
<td>2680-1434</td>
</tr>
<tr>
<td>3</td>
<td>Completely Regressed Immature Follicle. A thin-walled vesicle remains with or without a solid pigmented mass of cells. Yolk mass is usually localized centrally and undergoing phagocytosis.</td>
<td>Completely Regressed Accessory Follicle. Degenerated follicle is sac-like in structure. Thca is thin; follicle with or without a solid mass of highly vacuolated pigmented cells.</td>
</tr>
<tr>
<td>3'</td>
<td>Completely Regressed Accessory Follicle. Degenerated follicle is sac-like in structure. Thca is thin; follicle with or without a solid mass of highly vacuolated pigmented cells.</td>
<td>2435-272</td>
</tr>
</tbody>
</table>

Unlike corpora atretica previously discussed in herpetological literature, a central yolk mass persists in practically all atretic immature follicles of Crotaphytus collaris (Stage 3; Fig. 3E). These tiny structures (0.1-0.3 mm in diameter) are prevalent from May through September. In this last stage of atresia the entire follicle or a large portion of it may possess a solid mass of pigmented cells (Fig. 3A). Because of their pigmentation, variable location and volume of yolk,
these structures may be confused macroscopically with corpora albicantia. Microscopic examination as seen in Fig. 3 allows these structures to be clearly differentiated.

With the failure to complete vitellogenesis and enlarge, accessory follicles become atretic after the ovulation of mature ova. Macroscopically, the follicles appear bright orange and vary in size from 3-5 mm during the April-June yolk deposition period. Seldom are there more than two of these follicles per ovary. Follicular atresia of this type has been described in other reptiles by Altland (1951), Betz (1963), Boyd (1940), Bragdon (1952), Crews and Licht (1974), and Goldberg (1970; 1973).

An early regressing atretic accessory follicle (Stage 1') is characterized by a highly flocculent and vacuolated ooplasm (Fig. 5B). The theca is hyperemic and greatly hypertrophied. The entire yolk mass exhibits an invasion of phagocytic leukocytes.

The atretic accessory follicle (Stage 2') exhibits a peculiar festooned layer of giant cells which presumably originate from the follicular granulosa (Boyd, 1940). The club-shaped appearance of this layer characteristically has distal vacuoles bulging with absorbed yolk and a capillary communicating to the vascularized theca. One animal (Fig. 2A, B) possessed follicles in both ovaries undergoing Stage 2' atresia. Little free yolk remains in the follicle after this stage.

Following the absorption of the yolk, Stage 3' aaf usually appear as sac-like structures distorted in shape with only a very thin theca (Fig. 2C). However, as seen in Stage 3 aif, a solid pigmented mass of cells may persist, being localized at some point in the degenerated thecal sac. This mass may be the result of the inability of phagocytic cells to digest the nucleoplasm of these follicles. As the thecal sac becomes reduced, the pigmented mass remains and resembles a corpus luteum. These structures could be confused macroscopically with regressed corpora lutaea or even corpora albicantia; however, they are identifiable by their vacuolated nature, greater size and bright orange pigment.

Atresia of accessory follicles apparently plays a major role in maintaining female sexual refractiveness to ovarian stimulation in the lizard, Anolis carolinensis (Crews and Licht, 1974); the precise mechanism is currently under review (Cuellar and Cuellar, 1977). Tinkle (1961) found that less than 10% of female Uta stansburiana possess atretic follicles and that possibly 8-10% of enlarged follicles undergo atresia. He attributed the difference between clutch estimates using enlarged follicles and eggs or corpora lutea to atresia. In Xantusia vigilis, Miller (1948) noted a high incidence of atresia in accessory follicles which limited clutch size. Telford (1969) stated that in one year's sampling of Takydromus tachydromoides, 22% of each size group examined possessed atretic follicles. He added that atresia was the fate of follicles which begin yolk deposition too late in the summer to complete the cycle. Hoddenbach (1966) noted atresia in Cnemidophorus sexlineatus ovaries, but gave no estimate on numbers or frequency, whereas, Burkholder (1969) found atretic follicles in 80% of C. tigris. In Sceloporus occidentalis, the frequency of atretic follicles was at its highest following the breeding season in

FIGURE 4. Histosections of Crota phy tus collaris ovaries: A. Longitudinal section of the left ovary of SET 220 collected 31 August 1972. Atretic immature follicles characteristic of Stage 1 are present. B. Longitudinal section of the right ovary of SET 206 collected 9 August 1972. Regressed structures are surrounded by developing immature follicles. See Fig. 2 for abbreviations.
The striking similarity between regressed atretic immature follicles containing pigmented cells (Fig. 3C) and corpora albicantia (Fig. 3D) may restrict the use of corpora albicantia counts as a standard index for estimating reproductive potential. Upon histological examination, the differences between these structures become apparent in as much as the former usually possess yolk masses of variable size and location. Macroscopically, they appear almost identical in size, color, and morphology. It is also evident upon macroscopic observation that a regressed atretic accessory follicle and a regressed corpus luteum look much alike. Here again, histological examination reveals that the two structures are quite different. Telford (1969) used color change as well as size to distinguish corpora lutea from corpora albicantia. These criteria do not

Reproductive Potential.—Ballinger and Schrank (1972) referred to reproductive potential as the total number of eggs produced by a female during a single reproductive season. Clutch size may be determined by counting either the number of yolked ovarian follicles, oviducal eggs, or corpora lutea (Tinkle, 1961). Telford (1969) recommended the use of corpora albicantia as an indicator of clutch size. For determining the potential in C. collaris, all of the above criteria were used (Table 4). The estimated mean clutch size was 6.4 based upon these counts. Since corpora lutea become corpora albicantia which probably persist throughout the life of the lizard, these structures may be used as an estimate of the total number of eggs produced and ovulated by an ovary. However, because of the ability of collared lizards to produce as many as two clutches per season, this method could not be applied to the estimation of clutch size in old animals. Clutch size estimates vary geographically in C. collaris and have been recorded in Arkansas, 6.4 (this study); Kansas, 5.8 (Fitch, 1970); New Mexico, 5.3 (Parker, 1973), and Texas, 7.2 (Hipp, 1977). There is also a strong correlation \( r = 0.64, df = 75; P < .05 \) between clutch size and SVL (Fig. 6) in Arkansas collared lizards.

The striking similarity between regressed atretic immature follicles containing pigmented cells (Fig. 3C) and corpora albicantia (Fig. 3D) may restrict the use of corpora albicantia counts as a standard index for estimating reproductive potential. Upon histological examination, the differences between these structures become apparent in as much as the former usually possess yolk masses of variable size and location. Macroscopically, they appear almost identical in size, color, and morphology. It is also evident upon macroscopic observation that a regressed atretic accessory follicle and a regressed corpus luteum look much alike. Here again, histological examination reveals that the two structures are quite different. Telford (1969) used color change as well as size to distinguish corpora lutea from corpora albicantia. These criteria do not
TABLE 4. Estimates of reproductive potential for *Crotaphytus collaris* from Arkansas (clutch size represents largest number recorded in each category).

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Yolked Ovarian Follicles</th>
<th>Clutch Size</th>
<th>Oviducal Eggs</th>
<th>Clutch Size</th>
<th>Corpora Lutea</th>
<th>Clutch Size</th>
<th>Corpora Albicantia</th>
<th>Clutch Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Range</td>
<td>x</td>
<td>1st 2nd</td>
<td>N</td>
<td>Range</td>
<td>x</td>
<td>1st 2nd</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>3-9</td>
<td>5.8</td>
<td>8</td>
<td>9</td>
<td>3-7</td>
<td>5.2</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>4-11</td>
<td>8.1</td>
<td>11</td>
<td>8</td>
<td>13</td>
<td>4.9</td>
<td>6.4</td>
</tr>
<tr>
<td>&gt;2</td>
<td>4</td>
<td>6-10</td>
<td>8.0</td>
<td>10</td>
<td>–</td>
<td>3</td>
<td>5-8</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*Preovipositional period
**Postovipositional period

apply to *C. collaris* because of the possibility of misinterpreting ovarian structures. It is likely that future investigations into lizard ovarian histology will detect corpora albicantia in more species and provide additional information about these atretic structures.

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LITERATURE CITED


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