Physiological changes during the ovarian cycle of the female rock lizard, *Agama atra* (Sauria: Agamidae)

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Seasonal morphometric and physiological changes associated with vitellogenesis in the female agamid lizard, *Agama atra* are described. Vitellogenic activity during August – September marked the onset of the breeding season. It is suggested that at least two clutches were ovulated during the breeding season. Large abdominal fatbodies deposited prior to the winter months were possibly utilized to meet metabolic demands during winter and the onset of vitellogenesis during early spring (August – September). Follicular growth and fatbody deposition coincided with low plasma cholesterol levels. The liver index and total plasma proteins did not show a clear seasonal pattern. Of the seven plasma protein fractions, Fraction 2 ('human beta-globulin' mobility) increased during vitellogenesis while the foremost migrating proteins ('human albumin' mobility) showed a compensatory decrease. Total plasma calcium levels increased during vitellogenesis. The oviducts remained hypertrophied throughout the breeding season, while progesterone levels increased following each ovulation cycle in the presence of corpora lutea and remained elevated until oviposition. The onset of the second vitellogenic cycle (December) coincided with high progesterone levels.

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Seisoenale morfometriese en fisiologiese veranderinge tydens dooiemeerlegging in die wyfie akkedis, *Agama atra* word beskryf. Dooiermeerlegging neem 'n aanvang gedurende Augustus – September en die moontlikheid van twee ovulasie-siklusse in 'n broeiseisoen word voorgestel. Vet, gestoor in die vorm van groot abdominale vetliggame, word moontlik gebruik om aan metaboliese behoeftes te voorsien gedurende wintermaande asook vir die aanvang van die eerste dooierneerleggingsiklus (Augustus – September). Lae plasmacholesterolvlakke is gemeet tydens follikulêre groei en vetneerlegging. Die lewerindeks en totale plasmaproteïenkonsentrasie het geen duidelike seisoenale patroon getoon nie. Van die sewe plasmaproteïenfraksies het Fraksie 2 (menslike beta-globulienmobiliteit) kwantitatief toegeneem terwyl die vinnig migrerende fraksies (menslike albumienmobiliteit) afgeneem het. Die totale plasmakalsiumvlakke het toegeneem tydens dooiemeerlegging. Die eierleiers bly in 'n toestand van hipertrofie dwarsdeur die broeiseisoen. Die progesteroonvlakke styg drasties na ovulasie en bly net hoog totdat die eiers gelê word. Die begin van die tweede dooierneerleggingsiklus (Desember) geskied in die teenwoordigheid van hoë plasmaprogesteroonvlakke.

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Introduction

Agama atra Daudin, 1802, the South African rock agama, is a common diurnal lizard found throughout South Africa, inhabiting rocky areas (FitzSimons 1943; Branch 1981). Like most of the agamid lizards, *Agama atra* are oviparous and show a well-defined breeding season (Fitch 1970; Van Wyk 1983). Oocyte development in female oviparous lizards is usually associated with the accumulation of nutritive material within the cytoplasm. This phase always precedes oviposition and is invariably marked by the presence of the soluble yolk precursor (calcium-binding lipophosphoprotein = vitellogenin) (Follet 1974). In order to produce these yolk materials, drastic reorganization of metabolic activity precedes and accompanies vitellogenesis. The presence of this complex (vitellogenin) in the plasma of a vitellogenic female lizard may therefore cause plasma levels of protein, calcium, lipids, phospholipids and phosphoprotein to be elevated above levels in non-vitellogenic females. The object of this study was to investigate some of the expected plasma changes associated with vitellogenesis and the functional significance of the morphological changes in the ovarian, fatbody, hepatosomatic and oviducal indices.

Methods

Collecting

Agama atra females were collected monthly throughout 1979 and 1980 along the Walker Bay coast, south-western Cape Province, South Africa (34°33′S/19°21′E). Only females with a minimum snout-vent length of 8 cm were collected (8,02 ± 0,57 cm) and transported to the laboratory within 48 h of capture. The lizards were anaesthetized with diethyl ether, their mass determined to the nearest 0,01 g and the snout-vent length recorded with a vernier caliper to the nearest 0,01 mm. Blood samples (0,1 – 1 ml), were obtained from the post caval vein with a 25 gauge injection needle. The samples were centrifuged at 1 000 r.p.m. for 5 min and the plasma stored at −20 °C until further analysis. The liver, fatbodies, ovaries, and oviducts were excised and weighed to the nearest 0,0001 g, to be expressed as an organ index using a corrected carcass mass [bodymass − (fatbody + liver + ovarian + oviducal mass)] in the calculations (organ index = organ mass/100 g corrected carcass mass) (Van Wyk 1983). Not all the females collected during the breeding season were synchronized in their reproductive condition. Ovarian conditions noted each month were previously reported (Van Wyk 1983). Following autopsy, the lizards were therefore grouped according to ovarian activity rather than the month in which collected (Table 1).

Table 1 Reproductive stages used to group female *Agama atra* during the breeding season (after Van Wyk 1983)

Reproductive stage	Description
(i) Winter condition	Hydration stage ovarian follicles (diameter: smaller than 3 mm); no oviducal eggs, corpora lutea or fresh remnants of corpora lutea
(ii) Pre- or early vitellogenic	Onset of vitellogenesis (diameter larger than 3 mm but smaller than 5 mm); no oviducal eggs, corpora lutea or fresh remnants of corpora lutea
(iii) Preovulatory I (Pre I)	Late vitellogenic follicles (diameter larger than 5 mm); no oviducal eggs, corpora lutea of fresh remnants of corpora lutea
(iv) Postovulatory I (Post I)	Hydration stage follicles (diameter smaller than 3 mm); presence of oviducal eggs and corpora lutea with apparent blood supply; no remnants of corpora lutea
(v) Late postovulatory (Post II)	Early vitellogenic follicles (diameter larger than 3 mm and smaller than 5 mm). Large shelled oviducal eggs and corpora lutea; no remnants of corpora lutea
(vi) Preovulatory II (Pre II)	Late vitellogenic follicles (diameter larger than 5 mm); no oviducal eggs or functional corpora lutea (poor blood supply); fresh remnants of corpora lutea (pigmented)
(vii) Postovulatory III (Post III)	Hydration stage follicles (diameter smaller than 3 mm); presence of oviducal eggs, functional corpora lutea and fresh remnants of corpora lutea
(viii) Postoviposition	Hydration stage follicles (diameter smaller than 3 mm); no oviducal eggs or functional corpora lutea, but a large number of corpora lutea remnants (corresponding to two clutches) present

Biochemical analysis

Total cholesterol was determined according to the micromethod of Mattenheimer (1971) using 10μl of plasma and a cholesterol standard (400 mg/100 ml) prepared from Chelester-5-ol-3β (Merck Chemicals). Total plasma calcium was determined by the method used by Lance (1976). The biuret reaction method described by Mattenheimer (1971) was used to determine the total plasma protein concentration. Plasma protein fractions were separated on cellulose acetate membranes using a Beckman microzone electrophoresis system. A human plasma sample was separated concurrently on each membrane in order to have a reference standard of protein mobilities during the electrophoretic run. However, it must be stressed that although lizard plasma protein fractions may have mobilities comparable to the human plasma protein fractions, it was not assumed to be the same protein. The radioimmunoassay (RIA) procedure, to determine the plasma progesterone concentration, was originally described by Youssefnejadian, Florensa, Collins & Somerville (1972) and adapted by Faure (1975). Only plasma samples of 100 μl were used, resulting in smaller sample sizes than other biochemical determinations. Progesterone antiserum raised in sheep was provided by Dr J.C. Morgenthal (Department of Animal Physiology; University of Stellenbosch). A standard curve ranging from 0,5 to 5 ng/0,2 ml was used to calculate the unknown progesterone concentrations (Van Wyk 1982). Accuracy of the assay was determined by extracting a standard sample (3 ng/0,2 ml) simultaneously with plasma aliquots and the measured concentration calculated as a percentage of the expected concentration. It was found that an overestimation of 12% could be predicted for each assay.

Results

Gonadosomatic index (Figure 1)

A dramatic increase in ovarian mass was evident during spring ($P < 0{,}0001$; August *vs* Preovulatory I). The presence of corpora lutea remnants together with large preovulatory stage ovaries and two batches of corpora lutea in the presence of small postovulatory stage ovaries suggested that at least two vitellogenic cycles occurred during the breeding season. The second vitellogenic cycle ($P < 0{,}001$; Postovulatory I *vs* Preovulatory II) appeared to occur during early December.

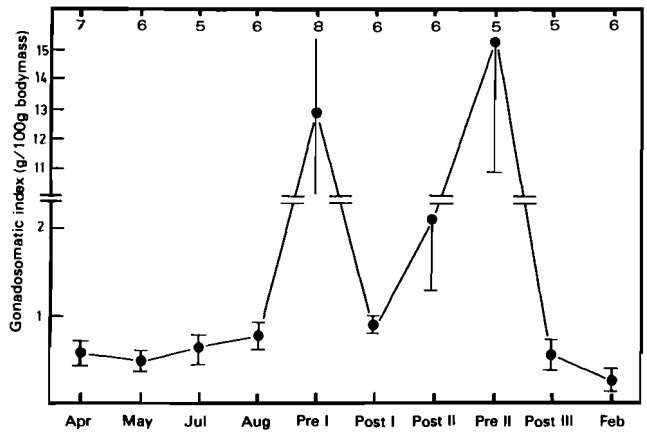

Figure 1 Variation in the ovarian mass, expressed as a gonadosomatic index (g/100 g body mass) during the ovarian cycle of the female *Agama atra*. Vertical bars indicate one standard deviation of the mean. The sample size is given above the bars. For explanation of reproductive stages see Table 1. (No lizards were collected during March and June.)

Small hydration stage ovarian follicles characterized quiescent ovaries outside the breeding season (February – August).

Fatbody index (Figure 2)

The abdominal fatbodies in female *Agama atra* increased significantly prior to the winter months and reached a maximum during April ($P < 0{,}001$; Postovulatory III *vs* April). Coinciding with the quiescent ovaries during the winter months was a reduction in the fatbody index. This reduction continued during deutoplasmic activity in the ovarian follicles to reach a seasonal minimum before oviposition of the second clutch. Although not significant ($0{,}1 > P > 0{,}05$; Postovulatory I *vs* Postovulatory II), some indication of fatbody deposition was observed during the second vitellogenic cycle. Occasional subcutaneous fatbodies in the pectoral region were also noted.

Total plasma cholesterol (Figure 3)

Similar to the fatbody index (Figure 2) the plasma cholesterol increased after the second oviposition cycle ($P < 0{,}001$; February *vs* May). Coinciding with fatbody utilization a significant decrease in the plasma cholesterol levels occurred during

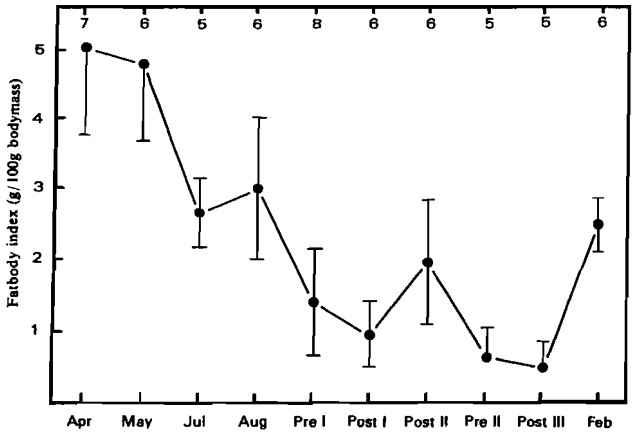

Figure 2 Variation in the fatbody index (g/100 g body mass) during the ovarian cycle of the female *Agama atra*. Symbols as explained in Figure 1 and Table 1.

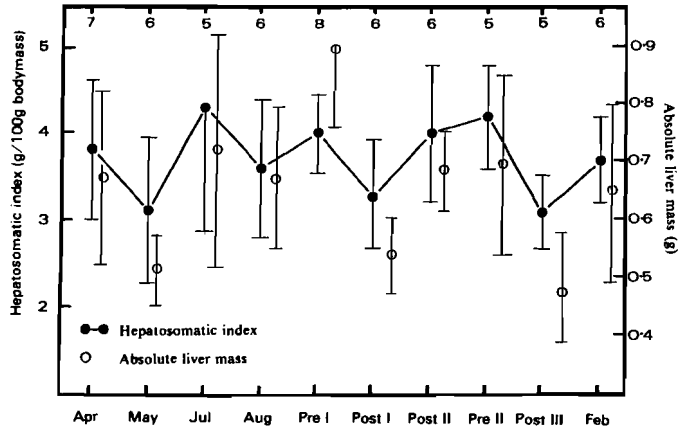

Figure 4 Variation in the hepatosomatic index (g/100 body mass) during the ovarian cycle of the female *Agama atra*. Symbols as explained in Figure 1 and Table 1.

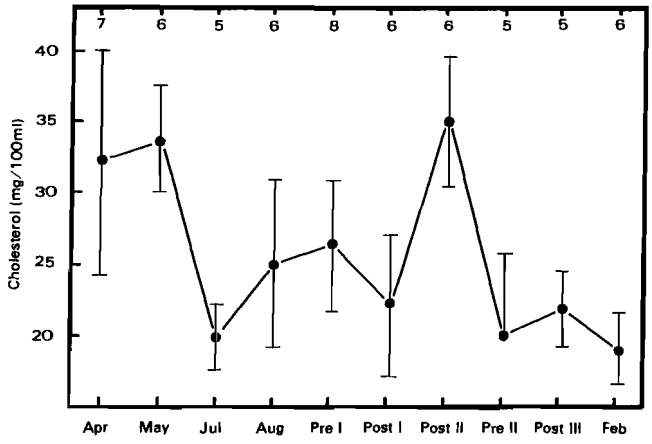

Figure 3 Variation in the total plasma cholesterol concentration during the ovarian cycle of the female *Agama atra*. Symbols as explained in Figure 1 and Table 1.

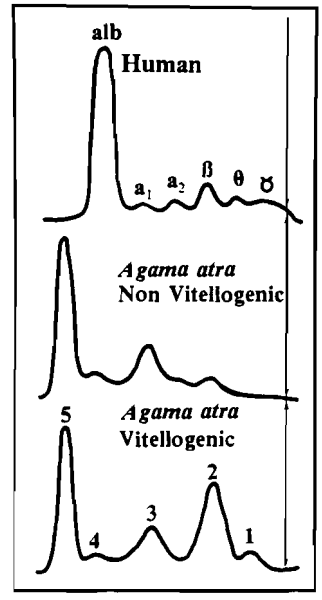

Figure 5 The separation of the plasma protein fractions upon cellulose acetate electrophoresis. Human plasma proteins (top), non-vitellogenic female *Agama atra* (middle) and vitellogenic female *Agama atra* (bottom). alb = albumin; a = alpha-globulin, β = beta-globulin; γ = gamma-globulin and θ = fibrinogen. The numbering of the *Agama atra* fractions is for convenience. Arrows indicate sample application points.

the winter months ($P < 0{,}001$; May *vs* July). After remaining relatively low during vitellogenesis (August to Preovulatory I) a significant increase was evident during the second vitellogenic cycle ($P < 0{,}002$; Postovulation I *vs* Postovulation II). Low levels were recorded prior to and during the onset of fatbody deposition.

Hepatosomatic index and absolute liver mass (Figure 4)

Considerable variation in the hepatosomatic index was evident throughout the year and no significant trends could be indicated. The absolute liver mass followed the same pattern throughout the year as demonstrated for the hepatosomatic index.

Total plasma proteins and plasma protein fractions (Figure 5 & Table 2)

The mean total plasma protein concentration reached its maximum during April (Table 2), followed by a continuous reduction until after the first ovulation cycle ($P < 0{,}001$; April *vs* Postovulatory I). A significant rise was evident during the second vitellogenic cycle ($P < 0{,}001$; Postovulatory I *vs* Postovulatory II), whereafter a reduction followed until after the last oviposition cycle.

The plasma proteins of female *Agama atra* were electrophoretically resolved into five major fractions (Figure 5). Fraction 1 showed very little mobility towards the cathode electrode and corresponded to the migration distance of the gamma-globulins present in human plasma. The mobility of Fraction 2 correlated with that of the human beta-globulins, Fraction 3 to that of the alpha-globulin in human plasma, the mobility of Fraction 4 corresponded to that of the human albumins, and the mobility of Fraction 5 exceeded the migration distance of human albumins. Table 2 summarizes the seasonal variation recorded in the quantitative concentration of each individual protein fraction. The slower migrating Fraction 2 did not change much outside the breeding season but a marked increase was evident during vitellogenic activity ($P < 0{,}001$; April *vs* Postovulatory I). The foremost migrating fractions (3, 4, 5), showed the same seasonal pattern and the inverse relationship to that of Fraction 2 during vitellogenesis was noteworthy.

Table 2 Variation in quantitative contribution of plasma protein fractions to total plasma proteins. [All values are given as mean and one standard deviation; values with similar alphabetic codes were compared using the Student *t*-test and only highly significant ($P < 0,001$) differences were noted]

n	Reproductive stages	Total protein (g/100 ml)	Plasma protein fractions (quantitative concentration) g/100 ml					
			1	2	3	4	5	2/5
7	April	4,32 ± 0,59	0,23 ± 0,04	0,80 ± 0,18[a]	1,03 ± 0,16[a]	0,55 ± 0,08[a]	1,70 ± 0,07[a]	0,47
6	May	4,16 ± 0,93	0,28 ± 0,06	0,95 ± 0,09	0,82 ± 0,07	0,46 ± 0,10	1,65 ± 0,05	0,51
5	July	3,78 ± 0,54	0,32 ± 0,06	0,81 ± 0,05	0,77 ± 0,06	0,33 ± 0,06	1,56 ± 0,10	0,52
6	August	3,13 ± 0,80	0,21 ± 0,07	0,89 ± 0,07	0,61 ± 0,20	0,23 ± 0,02	1,19 ± 0,08	0,74
8	Pre I	3,40 ± 0,90	0,25 ± 0,03	1,12 ± 0,10[a]	0,63 ± 0,13[a]	0,28 ± 0,10[a]	1,14 ± 0,10[a]	0,98
6	Post I	2,74 ± 0,47	0,23 ± 0,06	0,66 ± 0,02	0,58 ± 0,10	0,24 ± 0,04	1,03 ± 0,07	0,64
6	Post II	4,08 ± 0,80	0,35 ± 0,07	1,29 ± 0,10	0,83 ± 0,10	0,42 ± 0,02	1,15 ± 0,08	1,12
5	Pre II	3,36 ± 0,06	0,26 ± 0,03	1,00 ± 0,04	0,65 ± 0,08	0,27 ± 0,05	1,19 ± 0,13	0,84
5	Post III	3,21 ± 0,77	0,21 ± 0,08	0,71 ± 0,05	0,71 ± 0,06	0,32 ± 0,03	1,26 ± 0,05	0,56
6	February	3,75 ± 0,56	0,32 ± 0,06	0,72 ± 0,05	0,85 ± 0,04	0,41 ± 0,05	1,45 ± 0,09	0,50

Total plasma calcium (Figure 6)

Outside the breeding months, the total calcium concentration showed little variation (February to July). Despite considerable individual variation a significant increase occurred during the first vitellogenic period ($P < 0,001$; July *vs* Preovulatory I), followed by a reduction ($P < 0,001$; Preovulatory I *vs* Postovulatory II) and a significant increase ($P < 0,001$; Postovulatory I *vs* Postovulatory II) during the onset of the second vitellogenic period.

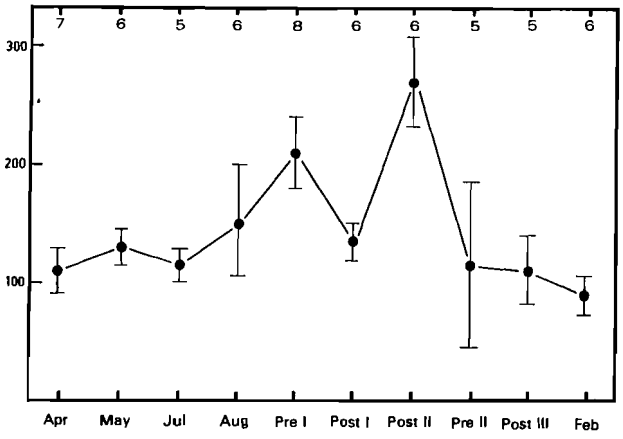

Figure 6 Variation in the total calcium concentration during the ovarian cycle of the female *Agama atra*. Symbols as explained in Figure 1 and Table 1.

Oviducal index (Figure 7)

Outside the breeding season the oviducts regressed in size and became thin collapsed tubules. Hypertrophy coincided with ovarian recrudescence and the highest index values were recorded during the postovulatory stages ($P < 0,001$; Preovulatory I *vs* Postovulatory I). The continued hypertrophy throughout the breeding cycle was noteworthy.

Plasma progesterone (Figure 8 & Table 3)

Like the oviducal index, little variation was evident outside the breeding season in the plasma progesterone concentration. Increased progesterone levels were only recorded after ovulation ($P < 0,001$; Preovulatory I *vs* Postovulatory I), followed by a decrease during the second preovulatory stage ($P < 0,001$;

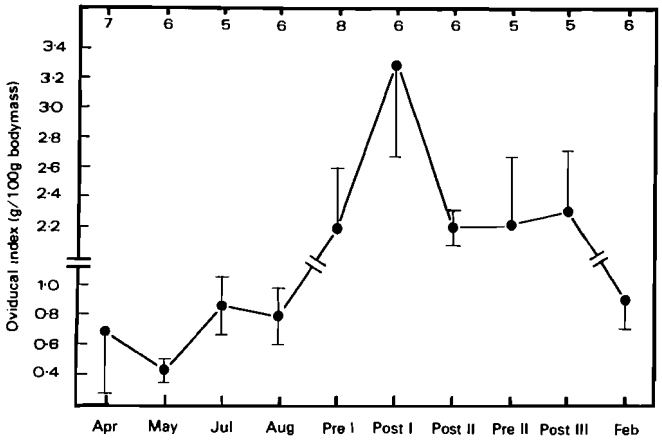

Figure 7 Variation in the oviducal index (g/100 g body mass) during the ovarian cycle of the female *Agama atra*. Symbols as explained in Figure 1 and Table 1.

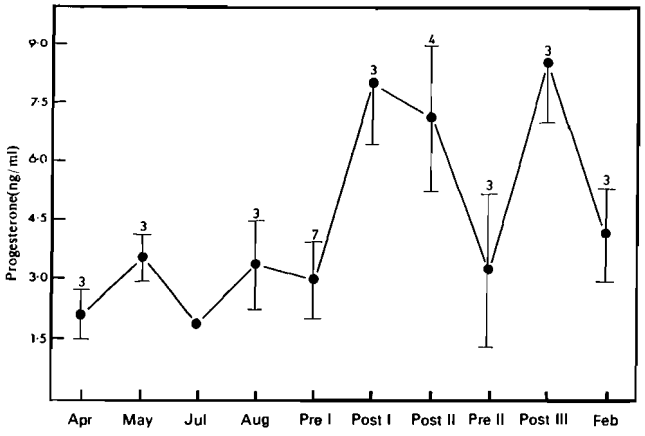

Figure 8 Variation in the total plasma progesterone concentration during the ovarian cycle of the female *Agama atra*. Symbols are explained in Figure 1 and Table 1.

Postovulatory II *vs* Preovulatory II). The second postovulatory stage was characterized by increased progesterone levels ($P < 0,001$; Preovulatory II *vs* Postovulatory III) followed by a rapid decline after the last egg-laying cycle ($P < 0,001$; Postovulatory III *vs* February). The high progesterone levels during the Postovulatory II stage coincided with the onset of the second

Table 3 Plasma progesterone levels (ng/ml) recorded during the reproductive cycles of reptiles cited from the literature. (Values given as mean and standard deviation)

Species	Stage of reproductive cycle				
	Previtellogenic	Preovulatory	Postovulatory	Late	
Chrysemys picta[1]	0,97 ± 0,06	5,01 ± 1,02	0,46 ± 0,01	–	Oviparous
Chelonia mydas[2]	0,17 ± 0,08	1,76 ± 0,13	0,68 ± 0,88	–	Oviparous
Chelonia mydas[2]	–	1,82 ± 0,29	3,00 ± 0,38	–	Oviparous
Chelonia mydas[3]	–	1,90 ± 0,50	2,36 ± 0,29	–	Oviparous
Chelonia serpentina[4]	0,25 ± 0,07	0,31 ± 0,11	1,44 ± 0,32	–	Oviparous
Agama atra[5]	2,14 ± 0,50	2,97 ± 0,99	7,45 ± 1,72	–	Oviparous
Naja naja[6]	± 1,4	± 3,00	10,5 ± 1,12	–	Oviparous
Uromastix hardwicki[7]	–	1,66 ± 0,30	13,41 ± 1,43	–	Oviparous
Chamaeleo pumilus[8]	0,86	0,94 ± 0,71	4,95 ± 3,90	2,30 ± 0,34	Viviparous
Sceloporus cyanogenys[9]	0,70 ± 0,15	0,90 ± 0,38	3,30 ± 0,48	–	Viviparous
Sceloporus jarrovi[10]	–	± 0,7	± 1,00	3,78 ± 0,38	Viviparous
Natrix taxispilota[11]	0,44 ± 0,04	0,01 ± 0,08	1,92 ± 0,24	–	Viviparous
Natrix sipedon[11]	1,27 ± 0,19	3,93 ± 0,83	4,950 ± 1,41	6,94 ± 0,78	Viviparous
Thamnophis elegans[12]	–	–	1,70 ± 0,30	6,20 ± 1,00	Viviparous
Thamnophis elegans[13]	–	3,30 ± 2,30	3,90 ± 0,50	–	Viviparous

References: [1]Callard & Lance (1977); [2]Lance & Callard (1978); [3]Licht *et al.* (1979); [4]Lewis *et al.* (1979); [5]Present study; [6]Bona-Gallo *et al.* (1980); [7]Arslan *et al.* (1978); [8]Veith (1974); [9]Callard *et al.* (1972); [10]Guillette *et al.* (1981); [11]Chan & Callard (1973); [12]Highfill & Mead (1975a); [13]Mead *et al.* (1981).

vitellogenic cycle (Figure 1). Table 3 records progesterone levels cited from other studies, in addition to levels measured in female *Agama atra*.

Discussion

Oviparous lizards occurring in the mid-temperate zone are usually characterized by longer breeding seasons and may produce up to six clutches during a single breeding season (Fitch 1970). Therefore it was not unexpected to find that at least two clutches were ovulated during the breeding season of female *Agama atra*. Seasonal breeding and the concomitant histological changes in the ovary of *Agama atra* have been described (Van Wyk 1983, 1984). During the period of vitellogenesis, preceding each ovulation cycle, selected oocytes of the female *Agama atra* undergo drastic size increments (Van Wyk 1984), which are similar to those reported for most non-mammalian vertebrates (Guraya 1978).

The importance of the mobilization of fat stores during vitellogenesis is known for most reptiles inhabiting the temperate zone (Derickson 1976). Food resources in temperate zones seem to be influenced by several environmental factors, such as photoperiod, temperature and precipitation. Derickson (1976) furthermore stated that food availability is the ultimate factor determining whether or not lipids are stored. The insectivorous nature of the *Agama atra* diet (Burrage 1973; Bruton 1976), metabolic demands during the winter months and the onset of vitellogenesis during early spring (Figure 1) predict that *Agama atra* store lipids prior to the winter months. Since the onset of vitellogenesis was only evident during early spring (Figure 2) it seems clear that fatbody lipids were utilized for metabolic demands during most of the winter months. The fact that the fatbodies remained reduced during the second vitellogenic cycle suggests that fatbody lipids were utilized mainly during the period prior to the first vitellogenic cycle. This also suggests that adequate food during the summer period made it unnecessary for *Agama atra* to store lipids (Van Wyk 1983).

Afroz, Ishaq & Ali (1971) showed that the fatbodies of the agamid lizard, *Uromastic hardwicki* contained a high proportion of cholesterol when compared to mammalian fat. In addition a decline in the cholesterol content during fatbody utilization was noted. Lance (1975) also suggested uptake of cholesterol by the fatbody in the cobra, *Naja naja*. Low plasma cholesterol levels coinciding with the early parts of fatbody deposition, reaching a maximum only after fatbody deposition in *Agama atra*, thus corroborate the reports by Afroz *et al.* (1971) and Lance (1975). Wiegand & Peter (1980) suggested that the major portion of ovarian cholesterol has an exogenous origin. Therefore, if ovarian uptake exceeds the cholesterol source as follicular growth proceeds, a decline in plasma levels occurs. The low blood cholesterol levels during follicular growth in *Agama atra* is in accordance with the Wiegand & Peter (1980) statement. Van Tienhoven (1968) stated that gonadotropin hormones may influence the permeability of the follicle wall, resulting in changes in plasma cholesterol levels. Since limited fatbody reserves existed at the onset of the second vitellogenic cycle in *Agama atra*, increased plasma cholesterol may rather be ascribed to increased cholesterol synthesis in the liver.

Neaves (1971, 1972) suggests that the ovarian yolk precursors have an extrafollicular origin. Liver hypertrophy during previtellogenic and vitellogenic stages was reported in several lizard species (Dessauer 1955; Telford 1970; Gerstle & Callard 1972; Yaron & Widzer 1978; Lin 1979). In spite of the above reports considerable individual variation was evident with the result that no clear seasonal trends in the liver index or the absolute liver mass were found in *Agama atra*. Callard, Banks & Banks (1972) pointed out that liver mass did not necessarily correlate with the synthetic activity of the liver in the lizard *Dipsosaurus dorsalis*. Oestrogen therapy in male *Agama atra*, however, resulted in significant liver hypertrophy ($P < 0,001$) (Van Wyk 1982) thus underlining the importance of the liver during vitellogenesis in spite of poor seasonal trends.

The synthesis and subsequent release of the yolk precursor vitellogenin (a complex calcium-binding lipophosphoprotein)

into the blood of the oviparous lizard during vitellogenesis is expected to have a marked effect on the composition of the blood chemistry (Follet & Redshaw 1968; Follet 1974). The total plasma protein concentration (TPP) in *Agama atra* did not show drastic seasonal changes but some coincidences with the seasonal trends in total plasma cholesterol were noted. It seems possible that the plasma cholesterol levels were a function of the uptake by the ovary and synthesis in the liver. Callard, Lance, Salhanick & Barad (1978) likewise reported no significant changes in the TPP during the annual ovarian cycle in the turtle *Chrysemys picta*. Callard *et al.* (1978) and Gapp, Ho & Callard (1979) suggested that seasonal changes in the plasma concentration of the individual plasma protein fractions may disguise significant trends in the TPP. In this regard a decrease in the foremost migrating fractions (especially Fraction 5) was evident in *Agama atra* during vitellogenesis. Plasma collected from vitellogenic *Agama atra* females was characterized by a significant increase in Fraction 2 ($P < 0{,}001$; April *vs* Preovulatory I and Postovulatory I *vs* Postovulatory II). Fraction 2 showed similar migration rates to the beta-globulins in human plasma. Similar mobilities were recorded in the lizards, *Anolis carolinensis* (Rosenquist 1969c from Dessauer 1974), *Dipsosaurus dorsalis* (Gerstle & Callard 1972) and numerous species from the snake genera, *Thammophis* and *Natrix* (Dessauer & Fox 1958, 1959) and turtles (Dessauer & Fox 1964). Migration rates corresponding to those of human gamma-globulins were recorded in the lizards, *Sceloporous cyanogenys* (Suzuki & Prosser 1968) and *Chamaelleo pumilus pumilus* (Veith 1974).

The calcium nature of the soluble yolk precursor complex in the blood seems to suggest an increase in the non-diffusible calcium component during vitellogenesis in oviparous lizards (Simkiss 1967). In the present study the highest recorded mean total plasma calcium level was noted during vitellogenesis. Likewise, Dessauer, Fox & Gilbert (1956) and Dessauer & Fox (1958) reported 30-fold increments in plasma calcium levels during vitellogenesis in two snake genera, *Thammophis* and *Natrix*. Similar trends were reported in the lizards, *Dipsosaurus dorsalis* (Gerstle & Callard 1972) and *Sceloporus cyanogenys* (Suzuki & Prosser 1968) and the cobra, *Naja naja* (Lance 1975). The effect of high plasma calcium levels during reproduction on the contractibility and heart rate in *Agama atra* is unknown. Henrotte, Cosmoss & Fenn (1960) indicated that the myocardium of the turtle *Pseudemys* was highly sensitive to rapid changes in plasma calcium. Possibly the concomitant increase in the level of the plasma protein Fraction 2 (possibly a β-globulin, known as the main binding site of calcium) may be responsible for maintaining low free calcium activity during reproduction. Considering the large number of cleidoic eggs being produced simultaneously in oviparous reptiles, Simkiss (1967) suggested that a large drain upon calcium reserves could be expected. The endolymphatic sacs of gekkonid and anoline lizards are known as calcium storage organs but no evidence could be found in *Agama atra* that such storage organs exist, neither could any conclusion be reached concerning secondary medullary bone formation prior to vitellogenesis as recorded in birds (Simkiss 1967). It appears that normal bone resorption might be sufficient to meet the calcium needs during the vitellogenic cycle of the female *Agama atra*. The same conclusion was reached for the turtles, *Sternothaenus adoratus* (Simkiss 1967) and *Pseudemys scripta elegans* (Suzuki 1963).

The seasonal oviducal hypertrophy recorded in *Agama atra* was in accordance with most of the reports dealing with lizards and snakes (Callard & Leathen 1967; Botte 1973a, b; Lance & Callard 1978). Callard & Klots (1973) suggested that the oviducal index could be used as an indirect indication of oestrogenic activity. Seasonal steroid cycles coinciding with oviducal growth in the turtles, *Chrysemys picta* corroborate this suggestion (Callard *et al.* 1978). Therefore, high oestrogen levels may be suggested at the onset of vitellogenesis in female *Agama atra*. During the same period a reduction in the granulosa layer and the disappearance of the large pyriform cells from the granulosa layer of the follicular wall was evident (Van Wyk 1984). The role of progesterone during hypertrophy and maintenance of the oviduct is not clear. However, it has been suggested that both oestrogen and progesterone are essential for oviducal maturation (Yaron 1972; Veith 1974). Elevated progesterone levels coincided with the hypertrophic state of the oviduct in *Agama atra*. In addition androgens are also reported to have a synergistic role (Botte, Granata & Christofaro 1974).

Table 3 shows that the plasma progesterone levels recorded outside the breeding season in *Agama atra* were much higher than the levels reported for other reptiles. However, the preovulatory levels did correspond to levels reported for the agamid lizard, *Uromastix hardwicki* (Arshan, Zaidi, Lobo, Zaidi & Oazi 1978). The postovulatory stage was marked by higher levels of plasma progesterone in *Uromastix* and the snake *Naja* than in *Agama atra*. High plasma progesterone levels and the presence of corpora lutea in *Agama atra* showing extensive vascularization throughout the thecal layer and the abundance of erythrocytes in the luteal mass, were always noted in the presence of oviducal eggs. Similar reports were noted for most oviparous lizards and snakes (Table 3). However, unlike in most squamates, Callard *et al.* (1978) reported a major progesterone surge prior to ovulation and the reaching of basal levels after oviposition in the turtles, *Chrysemys picta* and *Chelonia mydas*. Lance & Callard (1978) pointed out that vitellogenic cycles of squamates and chelonians differ considerably although the hormones controlling the process are probably similar. Several studies report the corpus lutea as the main source of plasma progesterone in reptiles (Highfill & Mead 1975a; Veith 1974; Arslan *et al.* 1978). Additional histochemical and hormonal assay investigations would be necessary in the oviparous lizard to substantiate this, as other sources of progesterone have been suggested. Callard & Leathen (1964), Huang, Vinson & Philips (1969), and Highfill & Mead (1975b) presented results indicating that the adrenals of snakes synthesize progesterone. However, Highfill & Mead (1975a) concluded that adrenals may be the major source at low levels of progesterone in non-pregnant snakes. The corpora atretica are mentioned as another progesterone source (Byskov 1978; Saidapur 1978; Gouder, Nadkarni & Rao 1979). However, Bona-Gallo, Licht, Mackenzie & Lofts (1980) suggested that the corpora artretica in the cobra *Naja Naja* made little contribution to circulating progesterone levels.

The functional significance of the corpora lutea and/or the high progesterone levels during the reproductive cycle of *Agama atra* remains enigmatic since the onset of the second vitellogenesis cycle in *Agama atra* coincided with high progesterone levels similar to those reported for turtles (Callard *et al.* 1978). Several investigators suggest that progesterone in one way or another inhibits the process of vitellogenesis (Callard & Klots 1973; Veith 1974; Klicka & Mahmoud 1977; Yaron & Widzer 1978). This was not observed in *Agama atra*, since the addition of progesterone to oestrogen treatment in the male

of this species did not prevent the appearance of vitellogenin in the blood (Van Wyk 1982).

In the light of the current state of knowledge concerning the reproductive physiology in oviparous lizards more elaborate investigations are needed before reaching conclusions. In this regard it seems evident that future studies on South African oviparous lizards and snakes could make an important contribution.

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