THE MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES DURING THE REPRODUCTIVE CYCLE OF THE FEMALE ROCK LIZARD, *AGAMA ATRA* DAUDIN, 1802

BY

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ABSTRACT

Morphological and physiological changes during the annual reproductive cycle of the female oviparous lizard, Agama atra, are described.

1. The onset of the breeding cycle was marked by vitellogenic ovarian hypertrophy (September) and ovulation of the first clutch occurred in October, after which a second clutch was produced. During February no vitellogenic females were collected.

2. The onset and termination of the breeding cycle, correlated with the spring and autumnal equinox as well as increased and decreased mean monthly air temperatures. Photothermal regimes (14L:10D and 25-45°C) induced vitellogenesis in females collected during the winter months.

3. Histological changes of the growing oocyte were studied. Oogonial proliferation, oogenesis and folliculogenesis in Agama atra appeared to be consistent with the general squamate pattern. Post-ovulatory and two types of atretic follicles are described.

4. The fatbody cycle showed an inverse correlation with ovarian hypertrophy.

5. Total plasma cholesterol varied between 200 and 350 mg·100ml⁻¹. A decrease was correlated with ovarian hypertrophy and fatbody depletion.

6. The total plasma protein concentration decreased during vitellogenesis and increased before ovulation. The plasma proteins separated into five major fractions upon cellulose acetate electrophoresis. Ovarian hypertrophy was accompanied by an increase in fraction II (similar mobility as human "Beta-globulins"). Fractions V ("albumin"), IV and III ("Alpha-globulins") were concomitantly decreased.

7. Plasma calcium concentrations increased significantly (P<0.001) during vitellogenesis.

8. A post-ovulatory progesterone surge (7450 pg·0.2ml⁻¹) with the presence of well developed corpora lutea. The onset of a second vitellogenic cycle was evident during relative high progesterone levels (1 500 pg·0.2 ml⁻¹).

9. The oviducal index showed a high correlation with ovarian development, indicating oestrogenic activity.

10. Oestrogen and oestrogen plus progesterone treatment resulted in increased plasma vitellogenic parameters in the male, Agama atra. Progesterone alone had no effect.

11. Both PMSG and HCG treatment (4 IU per lizard) induced vitellogenesis in females containing quiescent ovaries, although PMSG was found to be more potent in inducing ovulation.

12. No significant effect of arginine-vasotocin and oxytocin on oviposition could be observed.

KEY WORDS: Squamata, Agama, Female breeding season, environmental, seasonal changes, ovarian histology, fatbody, cholesterol, plasma proteins, calcium, oviduct, progesterone, vitellogenesis, gonadotropins, oviduct contractility.
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CHAPTER 1

GENERAL INTRODUCTION

The reproductive biology of South African lizards has not received much attention. Mostly brief field notes have been given by taxonomists about the number of young or eggs and the time of the year when pregnant females were collected (FitzSimons 1943). General life-history studies undertaken on South African reptiles contain superficial information on the reproductive biology (Broadley 1967; Burrage 1973; Haacke 1975). Veith (1974) reported on the reproductive biology of the ovoviviparous lizard, Chamaeleo pumilis pumilus with special reference to the role of the corpus luteum and progesterone. However, no detailed description of the seasonal morphological and physiological changes was given.

_Agama atra_ Daudin, 1802, commonly known as the South African rock agama, is a common diurnal lizard found throughout the Cape Province from sea level to mountain top, inhabiting rocky areas (FitzSimons 1943; Branch 1981). In his extensive taxonomic review of the South African lizards, FitzSimons (1943) provided brief field notes on _Agama atra_ and Oelofsen (1973 unpublished) noted additional ecological information. Burrage (1973) studied spatial and social organization in _Agama atra_ populations in the vicinity of the Walker Bay coast. Bruton (1976) described field and laboratory observations on feeding, social behaviour and thermal preferences of _Agama atra_. He concluded that _Agama atra_ is mainly insectivorous, ants being the main food of the juveniles, and larger insects that of the adults. Furthermore, this species is a typical heliothermic ectotherm, capable of selecting optimal thermal zones, using behavioural thermoregulation. A few studies on the biology of closely related species have been reported, namely Cowles (1956) on _Agama atricollis_ in South Africa, Harris (1964), Chapman and Chapman (1964) and Eyeson (1971) on _Agama agama_, elsewhere in Africa.
Most of the agamids are oviparous and show a well defined breeding season (Fitch 1970). The majority of the research, concerned with the role of environmental factors in determining the onset and termination of the breeding cycle, investigated parameters such as photoperiod, air temperature and nutrition. Moreover, the influence of these parameters is usually reported for male reptiles whereas only a few reports deal with the female reproductive cycle (Licht 1967).

A considerable body of literature describing the general ovarian cycle of squamates exist (Fitch 1970), but detailed ovarian histology has been described only for a few species. (Boyd 1940; Betz 1963; Varma 1970; Lance & Callard 1978; Callard et al 1978). Lance & Callard (1978) pointed out that the morphological changes occurring throughout the annual breeding cycle of squamates have been largely neglected. Despite the large number of studies made on reptiles there have been very few reports that relate seasonal changes in plasma constituents to the reproductive cycle. (Lance 1975, 1976; Lance & Lofts 1977, 1978).

One of the most characteristic changes is the appearance of vitellogenin (calcium-binding lipophosphoprotein) in the blood, acting as a yolk precursor. (Pollet 1974; Lance 1975; Arslan et al 1978; Callard et al 1978). Seasonal changes in plasma progesterone, correlated with the morphological as well as physiological changes, have only been reported for a few squamates. (Callard et al 1972b; Chan et al 1973; Veith 1974; Highfill & Mead (1975). Since the corpus luteum of at least some oviparous species produce progesterone, questions regarding the role of this hormone in oviparous reproduction could be raised.

The physiological roles of reptilian pituitary gonadotropins, especially in squamates, may differ considerably from those observed in mammals (Licht et al
1974b). Contradicting results on the influence of gonadotropins on follicular growth and ovulation in squamates are abundant. In the present study only preliminary experiments were performed in order to elucidate this problem.

The aim of the present study was to investigate the seasonal reproductive cycle, the associated changes which occur in ovarian histology and certain blood constituents. The information gained may help to fill the current gap in knowledge in the reproductive physiology of oviparous squamates. However, the present study was undertaken in the Fynbos Biome, which receives increased attention because of the uniqueness and zoogeographic importance of this region (Kruger 1978, 1979).
CHAPTER 2

SEASONAL STUDY: STUDY AREA AND BREEDING SEASON

2.1. Introduction

The adaptation of an animal to its particular ecological niche causes various adjustments in the timing of its reproductive efforts and in the number of offspring produced (Fitch 1970). Geographic locality and climate are known determining factors for the patterns of reproductive cycles in reptiles. In the present investigation, as in most previous reports, attention has been focused on photoperiod, rainfall and temperature. Other parameters such as humidity, cloud cover, windspeed and nutrition may be relevant. It is, however, difficult to obtain sufficient information regarding these factors and they are presumably closely related to the major variables.

In his classical work on the reproductive cycles of lizards and snakes, Fitch (1970) suggests that arid-temperature zone lizards may produce up to five or six clutches per season. Furthermore nearly all the agamids are oviparous and most show evidence of a well defined breeding season.

The objective of the present study was to demarcate the breeding season and to determine the number of ovulation cycles and thus the number of clutches produced in a breeding season. Furthermore, an attempt was made to correlate environmental parameters with ovarian activity in order to gain more information on possible environmental "Zeitgebers".
2.2. Procedure

2.2.1. The study area

Agama atra females were collected monthly throughout 1979 and 1980 along the Walker Bay coast (34° 33'S and 19° 21'E). Weather data was obtained from a weather station of the Department of Forestry situated in the study area.

2.2.1.1. Annual rainfall

Rainfall was recorded continuously throughout the study period and presented as total rainfall per month. The number of rainy days in each month was also recorded.

2.2.1.2. Photoperiod

Photoperiod was calculated using the formula of Van Leeuwen (1981):

\[
T = 0.988 + 30.3 (D + 30.3 (m-1)) \\
Z = 23.5 \cos (T + 10) \\
D = \frac{\text{arccos} \ (\tan (Z) \times \tan (b))}{7.5}
\]

T = Time of the year \\
Z = Decline of the sun \\
D = Day (1,2 ...... 30,31) \\
M = Month (1,2 ...... 11,12) \\
b = Latitude (minutes and seconds as a decimal fraction).

A negative value was applied to southern latitudes. The daylength values were accurate within 15 minutes.

2.2.1.3. Air temperature

Daily maximum and minimum air temperatures were recorded with a drumtype thermohygrometer in a Stevenson's screen (1.5 meters above ground level). Wet and dry bulb temperatures were used to calculate the daily relative humidity.
2.2.2. Collecting procedure

The lizards were mostly caught while basking in the sun on the rocks and collecting was, therefore, undertaken throughout the day during summer and during the midday period in the winter months. Before capture, conspicuous characteristics were noted with the aid of binoculars in order to determine the sex of the lizards. The characters included colour patterns, bodyform, shape of head, and the width of the tail base. Following capture, pressure was applied to the latero-ventral side of the tail in the vicinity of the cloaca in order to evaginate the hemipenis if present. Only females with a minimum snout-vent length (S.V.L.) of $8.02 \pm 0.57$ cm were collected to ensure sexual maturity.

Several collecting techniques were tested in the field. A glassfibre fishing rod (3 meters) fitted with nylon cord and a noose was successfully used in mountainous areas on windless days. In areas where crevices were abundant, nylon netting (1 square meter and 30 mm mesh) was used to close off the crevices into which the lizard retreated, thus catching it on emergence. In some cases large tweezers were used to extract lizards from crevices. The "chase and grab" method was also occasionally employed. The lizards were subsequently transported to the laboratory in either nylon bags or polyethelene cages.

2.2.3. Autopsy procedure

Six females were collected each month and transported to the laboratory within 48 hours of capture.

The lizards were anaesthetized with diethyl ether, weighed to the nearest 0.01 gram and the snout-vent length recorded with a vernier caliper to the nearest 0.01 mm. In order to obtain a bloodsample, the post caval vein was
cannulated with a 25 gauge injection needle under a Reichert stereomicroscope, and subsequently bled (0.1 ml - 1 ml). The blood samples were then centrifuged at 1000 r.p.m. in an Eppendorf clinical centrifuge. The plasma was stored at -20°C until further analysis.

The liver, fatbodies, ovaries and oviducts were excised and weighed to the nearest 0.0001 gram. The liver and fatbodies were subsequently fixed in 10% buffered formaldehyde whereas the ovaries and oviducts were treated as follows: the ten largest ovarian follicles were measured to the nearest 0.01 mm with an ocular micrometer attached to a Reichert stereomicroscope. The right ovary and right oviduct were then rapidly frozen on dry ice and stored at -20°C, while the left ovary and oviduct were fixed in Smith's fixative (Humanson 1967) and 10% buffered formaldehyde respectively.

In order to calculate an organ index (g. 100g bodymass⁻¹), a corrected carcass mass was computed into the calculations. The correction was calculated as follows:

1. Corrected carcass mass = Bodymass - (Fatbody mass + Liver mass + Ovarian mass + oviducal mass).

2. Organ index = \( \frac{\text{Organ mass}}{\text{Corrected carcass mass}} \times \frac{100}{1} \)

2.2.4. Reproductive condition

Following autopsy, Agama atra females were grouped into reproductive stages using as criteria the ovarian index, diameter of the five largest follicles of each ovary, presence of oviducal eggs, corpora lutea and remnants of corpora lutea. The general appearance of the five largest follicles was also noted and a final decision on the follicular stage was made only after histological investigation.
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: Presence of deutoplasmic yolk; follicle size larger than 3 mm but smaller than 5 mm; no oviducal eggs, corpora lutea or fresh remnants of corpora lutea.

iii. Pre-ovulatory I (Pre I): Late vitellogenic follicles (diameter larger than 5 mm); no oviducal eggs, corpora lutea or fresh remnants of corpora lutea.

iv. Post-ovulatory 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 post-ovulatory II (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. Pre-ovulatory 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. Post-ovulatory 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. Post oviposition (Feb): Hydration stage follicles (diameter smaller than 3 mm); no oviducal eggs or functional corpora lutea, and a large number of corpora lutea remnants (corresponding to two clutches).

In the presentation of the data for the ovarian cycle, these stages are presented as percentages of the monthly sample.
2.2.5. Effects of photoperiod and temperature on ovarian and oviducal recrudescence

2.2.5.1. Collecting and housing
Mature females (S.V.L. 8.10 ± 0.68 cm) were collected in the Clanwilliam district (19°9'E and 32°21'S) during the winter months (June and July 1980). Lizards were marked on the base of the tail with non-poisonous enamel paint and housed in a microhabitat, constructed with sand and rocks inside a controlled environmental room. The lizards were fed on Tenebrio molitor larvae and received water ad libitum.

2.2.5.2. Photothermal regimes
Lizards were exposed to various photothermal regimes ranging from winter to summer conditions (figure 1). Simultaneously temperature gradients (40°C – 20°C ambient temperature) were created on the rock piles using IR 240 – 250 Watt infra-red lamps. The illumination period of these lamps was separately controlled (figure 1). Control lizards were collected before and after the experiment from the study area.

2.2.5.3. Surgical procedure
Following cold-anaesthesia (0°C) the reproductive condition of the lizards was examined through a right latero-ventral incision (5 mm) in the abdominal wall. A chlorhexidine-alcohol solution (1 part chlorhexidine gluconate, 5% MV, "Hibitane", and 1½ parts distilled water made up to ten parts with absolute alcohol) was used as a disinfectant during all surgical procedures. After measuring the largest follicle diameters, the incision was closed with surgical thread and covered with an antibacterial powder ("Polyotic" SA gyarnid and a silicone topical protective ("Rikerspray"). Measurements were repeated after 16 and 28 days, after which the experiment was terminated.
FIGURE 1
Experimental photothermal acclimatizations in order to stimulate vitellogenesis in non-breeding female Agama atra.
2.3. **Results**

2.3.1. **Description of study area**

2.3.1.1. **Geographic location**

The Walker Bay coast in the vicinity of De Kelders (34°33'S and 19°21'E) is characterized by rocky cliffs, mostly of the Table Mountain series. These cliffs are typically composed of sandstone, quartzite, conglomerate and shale, often seen as a sequence of steplike ledges. To the north along the coast, calcareous dune sand of the Bredasdorp beds form sheer cliff faces seawards but with greater overhanging ledges.

Agama atra is common on the bare rock surface of the coastal cliffs (155 adults per hectare) and occurs sparsely on isolated bare, flat rocky outcrops surrounded by open shrub (Burrage 1974).

The study area lies in the winter rainfall area of the coastal western Cape and shows the typical Coastal Macchia (Coastal Fynbos) vegetation consisting of shrubs, grasses and other annuals (Acocks 1953).

2.3.1.3. **Rainfall**

The total monthly rainfall for 1979 and 1980 is presented in figure 2 which shows a rather erratic rainfall occurrence. The highest monthly precipitation (124 mm) was recorded in October 1979. However, when compared to that of 1978 and 1980 it indicated an abnormal high rainfall during this month. The expected maximum monthly precipitation would be, in the order of 80 mm (June - July), while the minimum was recorded during April (1.5 mm). The number of rainy days during 1979 indicated the typical winter rainfall pattern (figure 2).
FIGURE 2
TOTAL rainfall per month at the study area from January 1979 to December 1980. Number above points indicate the number of rainy days recorded for each month.
2.3.1.3. Air temperature and relative humidity

The mean monthly maximum and minimum air temperature (1979 - 1980) are presented in figure 3. The highest maximum monthly temperature was recorded during February 1979 (25.85 ± 4.31°C) and the lowest during July 1979 (17.71 ± 3.58°C). The highest minimum monthly temperature was recorded in January 1979 (18.50 ± 3.03°C) and the lowest occurred in July 1979 (8.23 ± 2.80°C). Extreme temperatures were recorded occasionally. (40°C in January 1979 and 3°C in July 1979). Looking at the degree of temperature change between consecutive months, it clearly showed that temperatures changed considerably during autumn and spring months (figure 4).

The relative humidity in the study area did not change considerably.

2.3.1.4. Photoperiod

The annual photoperiod for the study area is presented in figure 5. The autumnal equinox was calculated to be during the period 14 March - 21 March. The shortest day (9 hours 40 minutes) was 20 June. The spring equinox occurred during the period 19 September - 26 September, and the longest day (14 hours 19 minutes) was 19 December. Noteworthy was that the greatest change in air temperatures correspond to the equinox periods and furthermore correspond with the onset and termination of the breeding season (figures 3, 4, 5 and 6).

2.3.2. Ovulation cycles

In the presentation of the data to illustrate the breeding season, the reproductive stages present are presented as percentages of each monthly sample. (Figures 6 and 7).

All females collected during the autumn and winter months (April - July) were in the pre-vitellogenic stage, therefore containing small hydration stage ovarian follicles (smaller than 3 mm; translucent). During August 17%
The mean monthly minimum and maximum temperatures measured at the study area from January 1979 to August 1980.
FIGURE 4

Histograms illustrating the degree of temperature change between consecutive months (using maximum temperatures) (TOP) and the temperature fluctuation between maximum and minimum temperatures (BOTTOM).
The annual change in photoperiod at the study area (34°33'S; 19°21'E).
of the females had ovarian follicles larger than 5 mm (yellow appearance). It is evident (fig. 6 & 7) that the peak in vitellogenesis occurred in September (80% of sample had large follicles) whereas in October only 34% of the females had large vitellogenic follicles. Furthermore, it was noteworthy that 67% of the females collected in October contained oviducal eggs together with large functional corpora lutea (good blood supply). It was therefore assumed that ovulation occurred during October in most females. Another noteworthy aspect was that 10% of the females collected during November showed signs of deutoplasmic yolk formation, although oviducal eggs and functional corpora lutea were present. Furthermore, large vitellogenic ovarian follicles together with fresh remnants of corpora lutea were observed in 50% of the females. It was therefore assumed that oviposition occurred in these females and that a second ovarian cycle was evident. During December the number of females containing oviducal eggs together with vitellogenic follicles increased to 25% whilst 50% of the females contained large vitellogenic follicles together with remnants of corpora lutea at this time. In January only 17% of the collected females had large vitellogenic follicles while 83% had large shelled oviducal eggs. The February sample did not have any females with oviducal eggs or vitellogenic follicles, although remnants of corpora lutea were present. It was noteworthy that the number of these remnants corresponded to two normal clutches (8 eggs per clutch).

From this data it may be assumed that two peak ovulatory periods occurred during early October and early December respectively.

2.3.3. Effects of experimental photothermal regimes on ovarian and oviducal recrudescence

Photothermal conditions (figure 1) in the microhabitat resulted in ovarian recrudescence in winter lizards.
The presence of ovarian follicles with diameters either larger or smaller than 5 mm presented as a percentage of the monthly sample were used to indicate the onset of vitellogenesis.
The presence of oviducal eggs and corpora lutea together with follicle diameters, presented as percentages of the monthly samples.
The influence of photothermal regimes (figure 1) on the follicle diameter of non-breeding females. Control I was collected in April and Control II in May. The number above the bars represents the sample size.
Moreover, a significant increase was recorded in the oviducal index: (P<0.0001 Control I vs Day 28) and likewise the mean diameter of the largest ovarian follicles increased (P<0.0001, Control I vs Day 28) (figure 8). It was noteworthy that follicles of a second control group in July did not differ significantly from the first control group in May (P>0.02 Control I vs Control II).

2.4. Discussion

2.4.1. Ovulation cycles and clutch sizes

Geographic locality and the climate are obvious determining factors for the patterns of reproductive cycles in reptiles. In the cooler parts of the temperate zone there is a short and well defined breeding season (spring-summer). In some of the more northerly occurring reptiles, the females may ovulate only biennially. The mid-temperature zone on the other hand is characterized by longer breeding seasons and oviparous lizards may produce up to five or six clutches per season (Fitch 1970). Moreover, most tropical and sub-tropical species have extended breeding seasons, which in some instances last throughout the year.

With the present study area located in the mid-temperature zone it was not surprising to find that Agama atra females produced at least two clutches. It must be stressed, however, that evidence for the production of multiple clutches during the breeding season can at this stage not be considered conclusive in view of the small sample sizes, although the presence of, and number of corpora lutea remnants clearly suggest at least two clutches during the breeding season of Agama atra.

The number of eggs produced in each clutch (10 - 14) by Agama atra was consistent with figures reported by FitzSimons (1943) (7 - 14) and Oelofsen (1973 unpublished) (9 - 10). Harris (1957) recorded from five to seven eggs
in a clutch of the lizard, *Agama agama* whereas no more than seven eggs were recorded for the same species by Chapman & Chapman (1964). Available information suggested that a clutch size of 2 - 6 eggs was the most frequent number for most oviparous lizard species, although clutch sizes of 7 - 15 have been reported for a few species (Fitch 1970). Furthermore, information presented by Fitch (1970) indicated that oviparous reptiles tend to be more prolific than viviparous species, implicating short intervals between successive clutches (17.5 - 30 days). The interval between two clutches in *Agama atra* is suggested to be between 30 and 60 days. Although not quite in the range reported by Fitch (1970), the interval was in accordance with suggestions made by Oelofsen (1973 unpublished). Further studies investigating a larger number of individuals at any given time may produce more conclusive results concerning the number of clutches and the interval between the clutches in the female *Agama atra*.

2.4.2. Rainfall as a "Zeitgeber"

Chapman & Chapman (1964) reported that *Agama agama* female breed during or just after the main rainy periods in Ghana. Similar observations for the same species were made in Nigeria (Harris 1956) and on the equator (East Africa) (Marshall & Hook 1960). The latter suggested that the climate played a secondary role in causing an increased abundance of animal protein food (insects) and hence providing adequate nutrition for reproductive activities. Fitch (1970) and Goldberg & Robinson (1979) postulated that seasonal nutritional factors may be primary regulators of reproductive patterns in the desert environment.

In the present investigation monthly variations in the rainfall did not show a significant correlation with ovarian cycle. The study area being located in a winter rainfall area seems to suggest, if anything, an inverse relationship between rainfall and reproduction. Seasonal variation in fatbody mass has been suggested as indicative of temporal patterns of good availability
(Derickson 1976). The inverse relationship between fat body mass and ovarian growth observed in the female *Agama atra* thus suggests a seasonal variation in the relative abundance of insect food. According to Saunders (1979) a high constant temperature and long daylength act together to avert diapause, whereas low temperatures and short daylength act together to induce it. The relative abundance of insects is therefore expected to be low during the winter months. However, more data is required before rainfall as a regulatory factor in the reproductive cycle of *Agama atra* can be excluded.

The apparent lack of a clear correlation between rainfall and the female reproductive cycle in the female *Agama atra* suggests photoperiod and temperature and possible cues for control of ovarian activity.

2.4.3. **Photothermal factors as a "Zeitgeber"**

Lizards are ectotherms and thus subjected to thermal features of the environment. Like most reptiles, lizards are strongly heliothermic, thus being heavily dependent on the exogenous heat sources. Generally their biological processes obey the Van't Hoff law and hence their activity decreases when temperature is lowered.

Photoperiodic changes may however play an indirect role in controlling reproductive activity as the thermal balance of the lizard is related to daylength. Moreover, in view of its constancy, photoperiod may be a more reliable environmental "Zeitgeber" for controlling reproductive activity. The preferred body temperature which the lizard tends to maintain when active may be of particular importance and in this regard the duration of the daily heating suggests a secondary influence of the photoperiod.

A decline in reproductive activity after the autumnal equinox was clearly evident in the female *Agama atra* (figure 5) whilst the mean maximum air
temperature dropped concomittantly, indicating that ovarian activity may also depend upon a certain thermal "threshold". Air temperature slightly lower than the highest monthly averages may act as a thermal "threshold" (Gorman & Licht 1974). Bruton (1976) reported that the daily activity of *Agama atra* started at temperatures ranging between 19 - 20° throughout the year. Evidently after the autumnal equinox decreased day-lengths reduced the opportunity for heating the lizard to preferred temperatures in the present study area. Moreover, field observations corroborate this suggestion, as the activity of *Agama atra* was restricted to the midday period during the winter months. Conversely, the mean maximum air temperature increased after the spring equinox and this coincided with the onset of ovarian hypertrophy.

Licht (1967a) observed a distinct photoperiodism in the male *Anolis carolinensis*. He postulates, however, that temperature may be the direct modifier of testicular recrudescence rather than photoperiod. This view corroborates the suggestion made for *Agama atra* in the present study, namely that photoperiod probably acts in an indirect way to facilitate the temperature response. On the other hand increasing daylength, thus longer periods at the preferred body temperature, may be an important environmental cue determining the onset of ovarian recrudescence ("photoperiod threshold"). However, it does not necessarily imply that the onset and the termination of the breeding season is controlled by the same factors.

Several authors (Licht & Pearson 1969, 1973; Tinkle & Irwin 1965) suggested a relative refractoriness to either light or temperature or both which then serves as a mechanism for preventing premature ovarian hypertrophy in lizards. In the present study however, winter quiescent *Agama atra* females showed ovarian hypertrophy under conditions simulating summer photothermal regimes. Nevertheless it may be that some refractoriness exists during the winter months in the female *Agama atra* and that this apparent reproductive insensitivity may be by controlling both factors.
2.5. **Conclusion**

1. The breeding season started during August whereas no vitellogenic females were collected during February.

2. The available information clearly indicated that at least two ovulation cycles occurred during the breeding season, i.e. early October and early December.

3. The apparent lack of a clear correlation between rainfall and the female reproductive cycle in the female *Agama atra* suggests photoperiod and air temperature as potential modifiers of ovarian activity. Evaluation of the importance of these environmental cues needs more elaborate laboratory experimentation.
CHAPTER 3

HISTOLOGICAL CHANGES DURING THE OVARIAN CYCLE

3.1. Introduction

The histology of the reptilian ovary has been described in detail for only a few species (Boyd 1940; Betz 1963; Goldberg 1970; Varma 1970; Blanc 1971; Neaves 1971; Hubert 1971, 1973; Lance & Lofts 1978; Laughran et al 1981). Histological changes occurring during the annual breeding cycle have been neglected in most of the previous investigations. Lance & Lofts (1978) mentioned the importance of correlating changes in blood constituents with ovarian histology.

Tokarz (1978a) pointed out that the potential fecundity of vertebrate females is ultimately dependent on oogonial proliferation (i.e. the number of oogonia produced by mitoses); oogenesis (i.e. the number of oogonia that begin meiotic differentiation into primary oocytes) and folliculogenesis (i.e. the number of primary oocytes transformed into primordial follicles).

Two basic patterns of oogonial proliferation are found in nonmammalian vertebrates, namely where it is limited to the larvae or embryonic period (Elasmobanchs, some bony fish and birds, Tokarz 1978a) and on the other hand where it is found in the mature adult animal (most fishes, amphibia and reptiles, Boyd 1940; Miller 1948; Franchi et al 1962; Varma 1970; Lance & Callard 1978). Discrete regions, known as germinal beds, contain oogonia and are found in the ovaries of most adult reptiles. The tuatara Sphenodon punctatus might be an exception because oogenesis may be complete after embryonic development (Franchi et al 1962) and oogenesis may vary with the stage of the seasonal reproductive cycle in the lizards, Xantusia vigilis (Miller 1948) and Sceloporus jarrovi (Goldberg 1970). The number of these germinal beds varies among species (Loyez 1906). Altland
(1951) reported seasonal changes in oogonial mitoses in the box turtle, *Terrapene carolina*. Oocytes in different stages of development are also present in the germinal beds of reptiles (Boyd 1940; Miller 1948; Varma 1970; Jones et al 1976; Lance & Callard 1978).

Folliculogenesis, when prefollicular cells derived from the germinal epithelium surround the primary oocytes, is reported for several reptilian species. (Boyd 1940; Miller 1948; Betz 1963; Varma 1970; Hubert 1971; Tokarz 1977; Lance & Callard 1978; Laughran et al 1981). Simultaneously a migration towards the ovarian stroma takes place (Boyd 1940; Betz 1963; Jones & Greek 1975; Tokarz 1977).

After the oocyte has left the germinal bed the follicular epithelium becomes multilayered in the ovaries of snakes and lizards and three types of cells are differentiated (Miller 1948; Betz 1963; Wilhoft 1963; Varma 1970; Hubert 1971, 1973; Olmo & Taddei 1974; Jones et al 1975a; Lance & Lofts 1978; Guraya 1978; Laughran et al 1981). In this regard the functional significance of the large flask-shaped pyriform cells found in the granulosa layer of the squamates is obscure.

The histologic changes during oogonial proliferation, folliculogenesis and subsequent follicular growth until ovulation are described for the female *Agama atra*.

In most reptiles postovulatory follicles are transformed into corpora lutea (Badir 1968; Fox 1977). The interior of this discharged follicle fills with hypertrophied cells of the granulosa. (Bragdon 1952; Varma & Guraya 1973). The structure of the reptilian corpus luteum is generally similar to that of mammals (Forbes 1961). The functional significance of the corpus luteum in oviparous lizards is obscure.
Histological changes in the degenerating ovarian follicle (atresia) have been described for all vertebrate classes. Follicular atresia is a common phenomenon in the majority of reptiles studied although extremely rare in Gekkonidae (Loyez 1906). Oocytes may become atretic at any stage of development although atresia is more frequent in larger vitellogenic follicles (Betz 1963; Guraya 1965; Varma 1970).

3.2. Procedure

3.2.1. Autopsy and fixation
Described in paragraph 2.2.

3.2.2. Sectioning and staining
Fixed ovarian tissues were subjected to standard paraffin wax embedding (56 - 58°C melting point). Occasionally vitellogenic ovarian material was processed through double embedding with methylbenzoate and paraffin wax (Humanson 1967). Serial sections (4 - 6 µm) were cut on a Spencer rotary microtome (American Optical) and stained with Harris haematoxylin and eosin and Mallory's trichrome or periodic acid stain (P.A.S.) with Harris haematoxylin as counterstain (Humanson 1967; Bancroft and Stevens 1977).

3.2.3. Linear measurements
Linear measurements of ovarian follicles and other histological parameters were carried out on the median sections through the follicles of each series. A Nikon-microprojector was used to measure follicular parameters to the nearest 0.1 units. The linear measurements were made on the mesovarial side of each follicle and a total of ten measurements were made of each parameter.
3.3. **Histological description (Results)**

3.3.1. **Gonadosomatic index and follicular diameter changes during the reproductive cycle**

The changes in ovarian mass during the reproductive cycle were expressed as a gonadosomatic index (G.S.I.) (gram. 100 gram bodymass⁻¹) and presented in figure 9. The variation in the mean diameter of the five largest follicles of each ovary is presented in figure 10.

A rapid increase in ovarian growth occurred in spring (P<0.001; August vs preovulatory I). After the ovulation cycle the ovary was characterized by small ovarian follicles and the ovarian index was significantly decreased (P<0.001; preovulatory I vs post-ovulatory I). Together with oviducal eggs, corpora lutea showed definite vascularization while follicle diameter and ovarian mass increased. The appearance of the ovarian follicles indicated that a second vitellogenic cycle had started. Moreover, the presence of corpora lutea remnants together with large preovulatory ovarian follicles also indicated a second vitellogenic cycle. The subsequent post-ovulatory stage showed ovaries with small follicles and the presence of two batches of corpora lutea. Small hydration stage ovarian follicles were present in the quiescent ovaries throughout the winter months.

3.3.2. **Ovarian stroma and germinal epithelium**

The ovary of *Agama atra* is covered by a simple squamous epithelium, the peritoneal epithelium. The ovarian elements include seasonally varying numbers of developing follicles, corpora lutea, atretic follicles and a single germinal epithelium interspread in ovarian stroma. Mostly limited ovarian stroma is present and consist of fibroblasts, collagen fibres and several small bloodvessels.
FIGURE 9

Variation in ovarian mass, expressed as a gonadosomatic index (gram. 100 gram bodymass\(^{-1}\)) during the reproductive cycle of the female *Agama atra*. Vertical bars indicate the standard deviation of the mean, and the number above indicates the sample size. The reproductive stages are described in par. 2.2.4.
Variation in mean diameter of the five largest follicles of each ovary during the reproductive cycle of the female *Agama atra* (mean ± SD).
The germinal epithelium (G.E.) consists of an elongated patch of cells on the dorsal surface of the ovary and appears to be thinner at the periphery than in the centre (figure 13). The one side of the G.E. is continuous with the ovarian peritoneal epithelium whilst the opposite side curls ventrally (figure 13). A multicellular ovarian stromal patch borders the G.E. on its ventral side however the staining properties and histological appearance makes the G.E. clearly distinguishable (figures 13 and 14). The areolated ovarian stroma contains round to oval cells with deeply stained nuclei (figure 13) whereas the G.E. stroma has denser appearance with small oval cells, dark staining cytoplasm and a prominent single nucleolus (figures 14 and 15). Moreover, the G.E. is marked by an abundance of different sized oogonia in the peripheral and surface areas. The oogonial cytoplasm stains lightly and has a granular appearance whereas the round nucleus is strongly basophilic with a vesicular appearance (figure 14).

The oogonia sink below the surface before undergoing transformation into primary oocytes and during the subsequent growth period move towards the ovarian stromal patch (figures 15 and 16). The cytoplasm of the growing primary oocyte stains darker than before and appears homogeneous. The nucleus stains deeply basophilic.

Approaching the ovarian stromal patch, G.E. stromal cells surrounded the primary oocyte to form a single layer of squamousal cells (figure 15). The primary oocyte then projects into the underlying multicellular ovarian stroma (figure 16).

The G.E. appears to be most active during the previtellogenic phase (figure 19). Although the G.E. in the preovulatory phase appears active, very few migrating primary oocytes were in the G.E. stroma (figure 18).
3.3.3. Developing ovarian follicles

3.3.3.1. The oocyte: 0.1 - 0.3 mm

Follicles of this size have recently emerged from the G.E. The round, vesicular nucleus is eccentrically placed in the lightly staining ooplasm. Initially a large number of stomal cells accompanies the enlarging primary oocyte (figure 17). These small cells show scanty cytoplasm and a single deeply basophilic staining nucleus, with a single nucleolus. The basement membrane is formed on the outer border of these layers of small cells and is consequently now termed the granulosa layer.

Large granulosa cells occur soon after the primary oocyte emerged into the ovarian stroma. The nuclei of these cells are larger (12.3 ± 0.001 µm) and appear vesicular with a prominent nucleolus (figures 20 and 21). At this stage two types of granulosa cells are distinguishable namely the larger intermediate cells and the smaller basal cells (figure 21). Occasional mitotic figures can be seen in the basal cells. The zona pellucida is poorly developed at this stage, whereas the theca is not differentiated and consists of a single layer of flattened fibroblasts.

3.3.3.2. The oocyte: 0.3 - 0.5 mm

The follicles are still surrounded by a large amount of stroma (figure 22). The ooplasm stains lightly and has a homogeneous appearance which signs of acidophilic granules (figure 23). The nucleus appears granulated with a single nucleolus. The granulosa layer (35.6 ± 4.51 µm) consists mostly of intermediate cells (12.3 ± 0.131 µm) and small basal cells. Occasional large cells (17.65 ± 2.47 µm) with extremely large nuclei do however occur (figure 23). The large cells are characterized by the lightly staining cytoplasm and a distinct vesicular, basophilic nucleus. The zona pellucida
is poorly developed (figure 23). Likewise the thecal layer forms a thin, non-vascular fibroblast capsule. Noteworthy, are the flattened granulosa and thecal layers on the an-mesovarial side. Follicles of this size and description were present in the ovarian stroma throughout the year.

3.3.3.3. The oocyte : 0.5 - 1.0 mm

The ooplasm at this stage is sparsely vacuolated. Characteristically the vacuoles are arranged in a concentric zone with the size of the vacuoles diminishing towards the periphery of the ooplasm. The central ooplasm zone is vacuole free. Some follicles, however, showed no vacuoles at all. The vitelline membrane is evident as a very thin membrane surrounding the ooplasm (figures 24 and 25). On the granulosa side of this membrane a thin colloidal layer is sometimes visible, namely the zona pellucida. A strong PAS positive reaction is usually evident, whereas in H & E preparations it is left unstained. The granulosa layer increased in thickness (52 µm) at this stage and consisted mainly of three distinct cell types. Large pyriform cells are more differentiated than in the previous stage and they are characterized by a distinct flask-shaped appearance with lightly stained cytoplasm and a large basophilic nucleus (14.8 µm). Occasionally pyriform cells with cytoplasm showing a strong PAS positive reaction can be observed between lightly staining pyriform cells and intercellular bridges are also evident between pyriform cells and ooplasm (of the oocyte) (figures 26 and 27). Intermediate sized cells (12 µm) with a similar appearance as the pyriform cells are present adjacent to the zona pellucida. Chromatin granules and a single nucleolus can be seen in the nucleoplasm of the vesicular nucleus (7 µm). Small, round basophilic cells containing large round nuclei are present against the basement membrane (basal cells) and also adjacent to the zona pellucida between the intermediate cells (apical cells) (figure 25). The theca is weakly differentiated, consisting of concentrically arranged fibroblasts. Follicles of this nature were present throughout the year.
3.3.3.4. **The oocyte : 1.0 - 1.5 mm**

The ooplasm is more heavily vacuolated over a larger concentric zone. Similar to the previous stage, the vacuoles diminish towards the periphery (figure 28). The central ooplasm appears denser than in the peripheral zones whereas frequent irregular strands of denser ooplasm extend towards the periphery. Extremely minute basophilic yolk spheres occur at the periphery and extend inwards among the vacuoles. Additional acidophilic inclusions are present in a narrow zone near the periphery of the oocyte (figure 29). There is a marked increase in thickness of the zone pellucida, which stains homogeneously acidophilic with H & E staining and reacted positive with PAS staining (figures 30 and 31). The zona radiata (striated layer) occurs between the vitelline membrane and the zona pellucida (figures 29 and 30). No intermediate stage was observed in smaller follicles examined. Although the dark striations are clearly distinguishable the zona radiata did not stain with either H & E or PAS. No significant change in the granulosa thickness (50 µm) is evident although the usual cell types are present. Noteworthy is the increase in the number of intercellular connections between pyriform cells and the oocyte. Moreover, an increase in PAS positive staining pyriform cells occurred. Basal and intermediate cells are abundant against the inner border of the granulosa.

The thecal layer shows a moderate increase in thickness although not distinct differentiation. Occasional small capillaries were evident in the thecal layer. Follicles of this size and description were present throughout the year.

3.3.3.5. **The oocyte : 2.0 - 2.5 mm**

Most of the ooplasm has a alveolar appearance, mainly because of the marked increase in the number and size of the vacuoles. The peripheral zone however is without vacuoles although acidophilic inclusions are evident adjacent
to the vitelline membrane. Moreover, small basophilic yolk spheres are present in the vacuolated zone (figure 31). The zona pellucida is much thicker than in the previous stage in follicles of the pre-ovulatory ovary, whereas the zona radiata appears to be thinner (figures 12 and 31). In similar sized follicles collected from typical quiescent ovaries (winter months) the opposite was evident, namely a rather thick zona radiata and thinner zona pellucida (figure 32). There is a reduction in granulosa thickness (37 µm) (figure 11) and noticeably few pyriform cells occur whereas signs of degeneration in the granulosa are present. Intercellular spaces occur as the result of the reduction of the cytoplasm of the large pyriform cells. The nucleus of the pyriform cell stains lightly and a decrease in intercellular connections occur. Small basal cells, however, are abundant near the inner border of the granulosa (figure 32). The theca has a typical undifferentiated appearance although small capillaries are frequently observed near the outer surface. The thickness of this layer did not change significantly (figure 11) and erythrocytes are abundant between the flattened on the an-mesovarial side of the follicle while the zona pellucida and radiata appear to be unchanged.

These were the largest ovarian follicles during the winter months. During the breeding season, however, they were found together with large vitellogenic follicles.

3.3.3.6. The oocyte: 2.5 - 3.0 mm

the ooplasm appears to be heavily vacuolated with strands of denser ooplasm extending outwards between the vacuoles. The peripheral zone of the ooplasm is mostly free of vacuoles and is dusted with fine basophilic yolk spheres. The occurrence of a densely arranged layer of similar yolk spheres adjacent to the zona radiata (figure 33) is apparent. Large basophilic deutoplasmic yolk spheres are abundant in a concentric zone next to the peripheral zone. The size of these yolk spheres diminish towards the periphery of the ooplasm.
Moreover, fine acidophilic yolk is present in the centre of the ooplasm. The zona radiata has increased in thickness and distinct striations are visible. The inner border of this zona is completely covered with fine basophilic yolk spheres (figures 34 and 35). The zona pellucida is equal in thickness to the zona radiata but thinner than in the previously described follicular stage (figure 12). The granulosa layer, however, is markedly reduced in thickness (6.7 ± 0.56 µm) (P<0.05) and few pyriform cells occur (figure 11). The granulosa appears thicker where pyriform cells are still present (figure 34). The cytoplasm of the pyriform cells is more granulated and stained PAS positive, where the nucleus is still vesicular. Structures in the granulosa, which may resemble remnants of pyriform cells undergoing autolysis, can be observed (figures 35 and 36). Intermediate cells are absent whereas small basal cells are frequently observed against the inner border of the granulosa. Strong PAS positive reaction may occur near in the outer border of the granulosa, especially around the small apical cells (figure 35). The theca increased in thickness (19.67 ± 4.51 µm) compared to the previous stage but no distinct differentiations are evident (figure 11). Increased vascularization occurs in the surface areas of the theca.

These follicles were only observed during the vitellogenic cycles of the breeding season.

3.3.3.7. The oocyte: 3.0 - 4.0 mm

Most of the ooplasm is vacuolated and the dense ooplasm in the centre of the oocyte is reduced. Large heterophilic stained deutoplasmic yolk spheres increase in number and extend towards the centre where the spheres diminish into fine yolk granules. Medium sized yolk spheres occur in the peripheral zone whereas fine yolk granules are sparsely arranged in a concentric zone next to the zona radiata (figure 36). The thickness of the zona radiata had not changed considerably when compared with the previous stage although the
perpendicular striations had increased. The zona pellucida is, however, markedly decreased in thickness (figure 12) and the granulosa layer is likewise decreased. No true pyriform cells with evident cytoplasmic connections occur and the granulosa consists of typical cuboidal cells with a basophilic, vesicular nucleus and scanty cytoplasm (figures 36 and 37). The thecal layer is reduced in thickness although large blood sinuses and an abundance of thecal erythrocytes are present (figures 36 and 12). Moreover, large bloodvessels can be seen in the epithelium covering the ovary.

These follicles were only observed during the vitellogenic cycles of the breeding season.

3.3.3.8. The oocyte: larger than 5 mm (preovulatory)

Large deutoplasmic yolk spheres, diminishing in size towards the periphery, filled most of the ooplasm (figure 38).

The zona radiata is reduced in thickness although the distance between the perpendicular striations appears to have increased. The zona pellucida is also reduced to a thin layer which is barely visible. The granulosa is likewise reduced to a single layer of squamous cells with strongly basophilic stained nuclei (figure 38). Although the thecal connective tissue is reduced, a greater vascularity is evident. Large blood sinuses occur in the outer layers of the theca.

3.3.4. Postovulatory follicles (Corpora lutea)

3.3.4.1. Stage I (early postovulatory)

The early corpora lutea are mostly seen together with small ovarian follicles. The irregular shaped and apparent collapsed corpora lutea consist mainly of connective and epithelial tissue (figure 39). Shortly after ovulation the
granulosa cells undergo rapid hypertrophy to eventually fill the central cavity. The thecal layer is much thicker than in the pre-ovulatory follicle and has a relatively homogeneous appearance. (Mostly fibroblast and collagen fibres). Two distinct multicellular layers, an outer (externa) and inner (interna) are recognized (figure 41). The outer layer appears to be more collagenous with only a few fibroblasts visible arranged radially rather than concentrically. The cytoplasm of the fibroblasts stained slightly and the nuclei stained moderately basophilic using the H & E staining combination. Blood vessels are abundant in the outer layer of the thecal and occasionally pigment granules are present. The border between the outer and inner layer is marked by capillary spaces containing erythrocytes (figures 40 and 41). The inner theca varies in thickness and appears to contain a greater number of cells with fibroblasts and collagen fibres arranged concentrically (figures 40 and 41). The fibroblast nuclei are elongated and stained deeply basophilic. The irregular inner border next to the lutein cells is marked by the presence of darkly stained fibroblasts which occasionally form clusters of concentrically arranged cells. In the vicinity of the inner border, capillaries and pigment granules are usually observed between fibroblasts. In addition the inner border is characterized by the presence of a colloidal substance reacting strongly PAS positive (basement membrane).

The hypertrophied lutein cells show large round basophilic nuclei containing one to three nucleoli and fine chromatin in the nucleoplasm (vesicular) (figure 42). The cytoplasm stained moderately with eosin and has a finely granulated appearance (PAS negative). Although cytoplasmic vacuoles are present, the cytoplasm does not show the typical highly vacuolated appearance. The lutein cells are arranged randomly with the intercellular spaces larger in the peripheral regions.
3.3.4.2. Stage II

Macroscopically the corpora lutea of this stage have a more rounded appearance than at the previous stage and were seen together with ovarian follicles where the onset of the second vitellogenic cycle is evident. The central cavity is mostly filled with lutein cells and the thecal layers are thinner with staining properties similar to the previous stage. The thecal stoma is characterized by the presence of the unusual concentric "whorls" in the vicinity of the inner border. Both thecal layers show greater vascularization than in the previous stage but the vessels appear smaller. Pigment granules are frequently observed and occasional granulocytes can be observed in the thecal stroma. The basement membrane between thecal layer and lutein cells show a strong PAS reaction. The lutein cells are crowded and the nuclei smaller and more variable in size, although still vesicular, similar to the previous stage.

Large blood vessels frequently occur within the lutein mass although blood vessels are mostly associated with connective tissue strands penetrating the lutein mass. Granulocytes also occasionally occur between the lutein cells.

3.3.4.3. Stage III

The corpora lutea are smaller than before and have a pigmented appearance. The absence of oviducal eggs and the presence of large vitellogenic ovarian follicles indicate that oviposition was completed not too long ago, while the second vitellogenic cycle is evident. The corpus luteum shows the morphological changes associated with cellular atrophy. The cytoplasm of the lutein cells is vacuolated whereas the nuclei are picnotic. Moreover, the lutein cells are interspersed in a fibrous network originating from the thecae. The theca interna shows large empty vacuoles and the basement membrane is hardly visible. The outer theca also shows signs of continued destruction and most of the capillaries have disintegrated, leaving a heterogeneous mixture
of broken erythrocytes.

Noteworthy is the presence of colloidal substance in some of the vacuoles and spaces showing a strong PAS positive reaction (figure 45).

3.3.4.4. Stage IV

Remnants of corpora lutea can frequently be observed long after oviposition has occurred, even in the presence of a second clutch of oviducal eggs. A thick, dense connective tissue capsule is present while thecae as such do not exist. Some lutein cell nuclei are interspersed in a matrix of fibrous connective tissue and colloidal substance (PAS positive). (Figure 46).

3.3.5. The atresia of oocytes

Atretic follicles were observed during all stages of the reproductive cycle. Follicular atresia is recognized by changes in the granulosa cells and the ooplasm. Generally two types of atresia occur, i.e. atresia occurring in hydration stage follicles and in vitellogenic follicles.

3.3.5.1. Hydration stage atresia (figures 47 to 51)

The amount of ooplasm is markedly reduced and did not stain strongly. The sparsely distributed ooplasm has a granular appearance and occasional aggregations are observed. Ameboid macrophages are abundant where phagocytic activity is evident in the ooplasm. The macrophage cytoplasm has a vacuolated appearance and contains ooplasmic granules. The nuclear envelope is strongly basophilic whereas the nucleoplasm stains lightly, containing a round basophilic nucleolus (figure 48). Both the zona pellucida and radiata are absent from the inner border of the granulosa layer and in the granulosa itself no signs of intermediate or pyriform cells are evident. The granulosa layer is polymorphic and contains large irregularly shaped cells with distinct vacuolated cytoplasm and a round basophilic nucleus with condensed chromatin. Mitotic figures are frequently observed in the polymorphic granulosa (figures 49 and 50).
Static macrophages attached to the inner border of the granulosa, occur. The thecal layer is markedly hypertrophied but remains separated from the granulosa by the basement membrane (PAS positive) (figure 45). The outer regions of the thecal layer consist mainly of undifferentiated connective tissue cells and blood vessels and erythrocytes are abundant. Strands of connective tissue and blood vessels penetrate into the granulosa layer. Most cells are abundantly distributed throughout the thecal layers usually near these blood vessels. Folding of the follicular wall does not seem to occur in small atretic hydration stage follicles, but larger atretic follicles clearly show wall folding.

The hydration stage atresia occurred mainly during the winter months (quiescent ovaries) and were only occasionally observed during the breeding season.

3.3.5.2. Vitellogenic stage atresia (Figures 52 and 53)
The single layer of cuboidal or squamous granulosa cells is hypertrophied and invades the yolk mass. The clear stellate cytoplasm of the granulosa cells is filled with various sized vacuoles containing deutoplasmic yolk. The nuclei are highly basophilic staining with chromatin granules in the nucleoplasm. Macrophages are frequently seen in the granulosa, where intracytoplasmic yolk spheres indicate active phagocytosis. Hypertrophy is also evident in the thecal layer although no distinct differentiation could be observed. Remnants of the PAS positive staining, zona pellucida are occasionally seen adjacent to the inner border of the hypertrophied granulosa. The large deutoplasmic yolk spheres are irregularly shaped showing eroded edges and they frequently occur inside intracytoplasmic vacuoles of cells. Ameboid macrophages are abundant between the yolk spheres where increased phagocytic activity is evident.
As atresia proceeds the granulosa become regose, with strands of thecal connective tissue cells and bloodvessels accompanying the luminal projections. After the yolk is completely phagocytized the atretic follicle collapse and progressive resorption of the granulosa cells takes place. Gradually the entire follicle becomes invaded by connective tissue and the atretic follicle is reduced to a small scar in the ovarian stroma.

Early vitellogenic and preovulatory stage atresia was commonly observed during the breeding season especially in the post-ovulatory ovary.

3.4. Discussion

3.4.1. Developing ovarian follicles

The seasonal ovarian cycle in the female Agama atra corresponds to that found in the typical temperate zone reproductive cycles described by Fitch (1970).

Oogonia found in most adult reptiles are located in one or more discrete regions known as germinal beds (Loyez 1906). Miller (1963) suggests that these beds (G.E.) are remnants of the embryonic ovary in the adult animal. The number of germinal beds varies among species, which may be one interacting factor controlling clutch size (Jones & Duvall 1978).

Each ovary of Agama atra contained a single germinal epithelium adjacent to a patch of medullary ovarian stroma. Similar observations were reported for the lizards, Hoplodactylus maculatus (Boyd 1940), Calotes versicolor (Varma 1970), Anolis carolinensis (Jones & La Greek 1975) and Lepidodactylus lygubris (Jones & Duvall 1978). Unlike the condition of these lizards, the germinal epithelia of the iguanid lizard, Sceloporus jarrovi (Goldberg 1972), the cobra Naja naja (Lance & Callard 1978) and the snake, Natrix rhombifera (Betz 1963) were found to be scattered in small irregular patches of cells in the surface epithelium of the ovarian stroma.
Two discrete germinal epithelium patches at either end of the dorsal surface of the ovary were reported in the lizards, *Xantusia vigilis* (Miller 1948) and *Leiologisma rhomboidalis* (Wilhoft 1963).

Although no conclusive results were obtained concerning seasonal changes in mitotic activity in the germinal epithelium (G.E.) of *Agama atra*, some workers reported an increase in mitotic activity during the previtellogenic stages of other lizards (Franchi *et al* 1962; Goldberg 1970; Lance & Callard 1978); Franchi *et al* (1962) indicated that oogonia of reptiles differentiate to primary oocytes as they begin entering meiotic prophase (mostly embryonic ovaries) and become arrested in the diplotene stage until just before ovulation (See also Boyd 1940; Arronet 1973).

The process of folliculogenesis (surrounding of the naked oocyte by stromal cells) is essentially the same in *Agama atra* as described for several reptilian species (Loyez 1906; Boyd 1940; Betz 1963; Varna 1970; Jones *et al* 1976). Concurrent with folliculogenesis, the oocyte migrates inward towards the stroma of the medulla, similar to the general migratory pattern reported by Boyd (1940) for Hoplodactylus, Betz (1963) for *Natrix* and Jones *et al* (1976) for *Anolis*. The prefollicular cells surrounding the oocyte do not undergo extensive proliferation until the follicle moves into the ovarian stroma (medulla) and is therefore consistent with observations by Tokář (1977) in *Anolis*.

The absence of primary oocytes and primordial follicles from the germinal epithelium during the vitellogenic cycle may suggest seasonal activity in the germinal epithelium, but more confirmatory evidence is needed to reach a final conclusion.
Several reports suggest that gonadotropins (FSH) may mediate oogonial proliferation, oogenesis and folliculogenesis either directly or indirectly through ovarian steroids. (Jones et al 1976; Tokarz 1978a). However, Tokarz (1978b) reported that oestradiol was ineffective in increasing the number of (3H) thymidine-labelled oogonia in Anolis carolinensis. Jones et al (1976) proposes that both the formation and maintenance of primary oocytes and primordial follicles are gonadotropin dependent in *Anolis carolinensis*. Eyeson (1971) on the other hand suggested that the maintenance of previtellogenic follicles in immature *Agama agama* was gonadotropin independent.

In the present investigation it was found that the differentiation of the granulosa layer was characterized by the appearance of three distinct cell types. Observations of the granulosa layer of differentiating follicles in the size hierarchy suggested that the large pyriform cells were derived from the intermediate cells which in turn were derived from the small cells. Although little information was obtained on the mitotic activity in the small cells the relative abundance of the three cell types suggested differentiation rather than mitotic activity. Similar suggestions were made for other squamates by Loyez (1906), Thing (1918), Betz (1963), Blanc (1971), Hubert (1971 & 1973), Neaves (1971), Lance & Callard (1978) and Laughran et al (1981). However, Braun (1977), Hoffman (1839) and Porta & Zahnd (1961) suggested that oocytes were incorporated into the granulosa layer to originate pyriform cells. Evidence to support their suggestion could not be found in the present histological study.

Hubert (1973) noted that the number of pyriform cells increased in the granulosa layer concurrent with initial follicular growth, although no mitotic activity occurred in the pyriform and intermediate cells. Betz (1963) essentially reported similar observations, although it was suggested that the mitotic activity arrested soon after differentiation of the first
pyriform cells. Therefore, pyriform cells differentiate solely from existing granulosa cells resulting in a decrease of cells in the granulosa. In contrast, Lance & Lofts (1978) reported that the small granulosa cells continue to divide until the onset of yolk deposition, thereby supporting the findings by Blanc (1971), Hubert (1971a) and Olmo and Taddei (1974). Filosa et al (1979) clearly indicated that the number of large cells increased during oocyte growth, concurrently with increased small cell members. The differentiation of large cells was arrested but small cell division continued. However, autoradiographic studies have indicated that small basal granulosa cells proliferate and give rise to intermediate and pyriform cells. (Jones & La Greek 1975a; Tokarz 1977, 1978b; Filosa et al 1979). Filosa et al (1979) also indicated that proliferation first occurred in the apical small cells after which migration towards the inside of the follicular epithelium occurs.

Characteristic of the flask-shaped pyriform cell found in the Agama atra ovary was the narrow protoplasmic prolongations which appeared to be confluent with the ooplasm (figure 27). Morphologically these cells correspond to previous reports by various authors. (Hubert 1971 a, b, 1973, 1976; Blanc 1971; Neaves 1971; Taddei 1972a; Varma 1970; Jones & La Greek 1975 ). The cytoplasmic prolongation is a remarkable feature of these cells since they represent an exception to the general rule in vertebrates, in that they represent the only clear example of intercellular bridges between follicular cells and the oocyte. Electron microscopic studies of ovaries from mammals, birds, amphibians and turtles indicated the absence of intercellular bridges thus making their occurrence in squamates exceptional (Neaves 1971).

In the Agama atra ovary the intercellular connections become more abundant in the granulosa layer of the larger hydration stage follicles (late previtellogenic). Similar results were reported in the cobra, Naja naja (Lance & Callard 1978), the lizards, Sceloporus jarrovi (Goldberg 1970),
Anoles carolinensis (Neaves 1971) and Lacerta sicula (Andreucetti et al 1978; Filosa et al 1979). Andreucetti et al (1978) illustrated that the intercellular bridge was established before the differentiation of the pyriform cells; therefore suggesting that intermediate and possibly basal cells possess an intercellular connection with the oocyte.

The functional significance of the cellular organization of the granulosa, especially the pyriform cells, remains obscure. It has been suggested that these cells are involved with yolk production (Loyez 1906; Betz 1963; Blanc 1970; Goldberg 1970; Varma 1970). However, electronmicroscopic observations of pyriform cells in Anolis carolinensis showed nothing resembling yolk in the pyriform cellbody or cytoplasmic bridges (Neaves 1981). Furthermore, no seasonal variation in the granulosa cells has been reported by these workers. In the present study it was however, observed that in large hydration stage (previtellogenic) follicles, the pyriform cytoplasm contained PAS positive granules. On the other hand small hydration stage follicles showed a typical pale pyriform cytoplasm as described by most workers (Boyd 1940; Betz 1963; Varma 1970). The PAS positive reaction found in the pyriform cells of Agama atra may be the result of the presence of glycogen and ribonucleoproteins to be exchanged between pyriform cell and ooplasm. However, no such movements can be deducted from microphotographs (Neaves 1971). Observations in Agama atra further indicated that the pyriform cells degenerate during the onset of vitellogenesis. Similar observations made by several workers (Neaves 1971; Hubert 1973; Taddei 1971; Lance & Callard 1978) therefore question the yolk production hypothesis.

Neaves (1971) reported microvilli at the basal surface of the pyriform cell which suggested the transport of extrafollicular substances. Corresponding to oocyte growth the follicle cells and oocyte surface develop very complex
processes as well as microvilli which are believed to increase the surface area for absorption of substances. (Hubert 1973; Blanc 1971; Rahil & Narbaitz 1973; Andreucetti et al 1978; Laughran et al 1981). Micropinocytotic vesicles between the basis of the microvilli clearly indicate massive absorption of substances from the follicular epithelium (Guraya 1978). Rahil & Narbaitz (1973) have observed dark glycogen granules distributed among the microvilli secretions from the follicular cells. The intercellular bridges between the follicle cells and the oocyte reported for the lizard ovary might be an important transport route (Neaves 1971; Hubert 1973; Taddei 1972a; Guraya 1978). These workers also reported conspicuous bundles of microtubules in the intracellular bridges which may indicate an intercellular transport system.

Neaves (1971, 1972) also suggested synthetic activity in the apical cytoplasm thus corroborating the hypothesis of extrafollicular exchange. Ultrastructural studies of follicle cells have revealed the presence of various organelles such as mitochondria, elements of granular endoplasmic reticulum, many free ribosomes, a well-developed Golgi complex, glycogenlike particles, annulated lamellae and microfilaments (Hubert 1973; Blanc 1971; Neaves 1972; Taddei 1972b; Andreucetti et al 1978; Laughran et al 1981).

Histochemical, ultrastructural and autoradiographic studies indicated that the follicular epithelium is very active in synthesis of RNA (especially ribosomes) proteins, glycogen and phospholipids (Blanc 1971; Taddei 1972b; Hubert 1973; Guraya 1978). The greater morphological development and diversity of the nucleolus in the pyriform nucleus are related to active ribosome synthesis. The ribosomes are abundant in the cytoplasm of pyriform cells and seem to migrate into the ooplasm through the cytoplasmic bridges (Guraya 1978). Lipid bodies, consisting of phospholipids, increased in numbers with the growth of the follicle indicating utilization for cellular construction (lipoprotein membranes) and other metabolic activities concerned
with the maturation of the follicular epithelium (Guraya 1978). Early workers like Hubert (1973) mentioned the possibility of chromatin material exchange between pyriform cell and ooplasm.

It can be concluded that the available information clearly suggests that the follicular epithelium and intercellular bridges may be responsible for the maintenance of the growing previtellogenic oocyte.

Seasonal changes in the thickness of the granulosa have been widely reported for squamates. Granulosa thickness correlated with follicular development in Agama atra is presented in figure 11. It is clearly evident that the granulosa layer increased in thickness during the previtellogenic stage corresponding to the increase in number of pyriform cells. The subsequent reduction of the granulosa appeared to be the result of pyriform cell degeneration concomitant with the onset of vitellogenesis. Simultaneously a reduction in the occurrence of intercellular bridges was observed. In the vitellogenic follicles, the pyriform cells were smaller and the follicular epithelium single layered and monomorphic, similar to previous descriptions by Betz (1963), Varma (1970), Blanc (1971), Hubert (1973) and Guraya (1978). Loyez (1906) described the disappearance of pyriform cells by the extrusion of their entire protoplasm, including the nucleus, through the intercellular bridge into the ooplasm. Similar observations came from Betz (1963) and Blanc (1971). Moreover, Betz (1963), Varma (1970) and Goldberg (1970) suggested that the pyriform cells may be unicellular glands with a holocrine function. These suggestions could however not be substantiated by observations in Agama atra. Boyd (1940) suggested that the pyriform cells undergo shrinkage resulting in a shrivelled cell membrane and the occurrence of intercellular spaces. Wilhoft (1963) and Neaves (1971) reported similar observations. Corresponding to the decrease in granulosa thickness and the disappearance of intercellular bridges was the reduction of PAS positive granules and the
FIGURE 11. (top) Variation in the thickness of the zona, pellucida and zona radiata during folliculogenesis.

FIGURE 12. (bottom) Variation in the thickness of the granulosa and thecal layer during folliculogenesis.
occurrence of empty cytoplasmic vacuoles. These changes may be indicative of the functional significance of the following epithelium to the growing previtellogenic oocyte.

The abundance of small granulosa cells following pyriform degeneration may indicate some regulatory activity which had no initial effect on the small granulosa cells. With further enlargement of the follicle, however, a single layered cuboidal granulosa developed and later a single squamous epithelium surrounded the oocyte. The fact that this characteristic reduction coincided with the onset of vitellogenesis (which may be used as an index of increased oestrogen secretion (Lance 1976) may suggest a steroidogenic regulatory action. However, FSH treatment in Anolis carolinensis resulted in a decrease in the number of pyriform cells as well as basal cells whereas the apical granulosa cells increased in numbers. (Jones & La Greek 1975). It was therefore suggested that the apical cells remained as the cuboidal granulosa layer which according to Jones et al (1974) showed steroidogenic activity. The limited information therefore suggests that oestrogen or gonadotropins may be responsible for the regression of the granulosa layer in the female Agama atra.

The zona pellucida is formed between the oocyte surface and the follicular epithelium in Agama atra which corresponds to the condition found in most lower vertebrates. During the early stages of oocyte growth the zona pellucida formed a homogeneous layer. Differentiation into an outer homogeneous, inner striated layer occurred with further ovarian growth. Similar reports came from Varma (1970), Jones & La Greek (1975a) and Guraya & Varma (1976). Previous ultrastructural studies moreover revealed that the oocyte surface forms many microvilli and simultaneously follicular cell processes are formed perpendicular to the granulosa surface. The microvilli and cell processes form the striated zone whereas electron-dense material deposited between the microvilli and cell processes form a homogeneous zona
pellucida adjacent to the granulosa layer (Hubert 1971, 1973; Neaves 1971; Guraya 1978; Laughren et al 1981). In *Agama atra* small yolk droplets present adjacent to the inner border of the zona radiata may indicate the accumulation of yolk precursor in the oocyte from extra-oocyte origin. Because the large preovulatory oocyte contained only large yolk bodies deep within the ooplasm, it can be assumed that the smaller vesicles adjacent to the granulosa layer probably fuse together to form the larger yolk bodies. Reports by Neaves (1971) and Dumont (1978) corroborate this suggestion. Several workers reported that the microvilli and cellular processes are greatly increased in size and number, thereby resulting in an increased zona radiata (Hubert 1971, 1973; Guraya 1978). The characteristic increase in both microvilli and cell processes may indicate an increased surface area for extra-follicular material exchange. In the present investigation the maximum thickness in the zona radiata during the vitellogenic and preovulatory stages was consistent with the above reports. Several other studies indicated a similar correlation between zona radiata thickness and vitellogenesis (Boyd 1940; Varma 1970; Lance & Callard 1978; Laughran et al 1981).

The zona pellucida reached maximum thickness in the vitellogenic stage in *Agama atra* which corresponds with reports by Boyd (1940) for *Hoplodactylus*, Varma (1970) for *Calotes* and Lance & Callard (1978) for *Naja*. Furthermore, the carbohydrate (PAS positive) nature of the zona pellucida in *Agama atra* is consistent with reports by Guraya (1978) (review). However, if the zona pellucida represents the accumulation of yolk precursor in the peripheral zone of the ooplasm as the result of endocytosis, it could be expected that the zona pelludica would reach its maximum during the preovulatory stages. This, however, was not the case in *Agama atra*. It is therefore evident that the functional significance of the zona pellucida remains obscure.
In the present study the surrounding stromal tissue forms a fibrous thecal layer, separated from the granulosa by a basal lamina around the ovarian follicle. Several workers reported a similar condition for several reptilian species. (Varma 1970; Guraya et al 1976; Blanc 1971; Jones & La Greek 1975; Tokarz 1977; Laughran et al 1981). The increase in vascularization of the thecal layers in large follicles in the Agama atra ovary is consistent with a report by Gerrard et al (1973) which indicated that larger follicles contained more blood per mass of thecal tissue than in small follicles.

The presence of mast cells in the thecal layer of maturing follicles in Agama atra and also previously reported in Anolis carolinensis (Jones & La Greek 1975b) may suggest a histamine effect on vessel size and permeability. However, Tokarz (1973) suggested that oestradiol is the principal factor affecting thecal vessel permeability and hyperemia.

The differentiation of the thecal layer into a fibrous outer and cellular inner layer is widely reported in squamates, but in Agama atra no significant differentiation was evident throughout the breeding season. The condition in Agama atra is consistent with observations in the cobra, Naja naja (Lance & Callard 1978), the lizard, Agama agama (Eyeson 1971), and the snake, Natrix rhombifera (Betz 1963). Likewise, Guraya (1965) noted that the theca interna showed no hypertrophy in large follicles in the snakes, Naja tripudians and Bungarus coenilens. In contrast with the present study and others, Boyd (1940) and Varma (1970) reported hypertrophy of the theca interna during follicular maturation.

3.4.2. Postovulatory follicles

Corpora lutea are found in a variety of reptiles, including turtles and oviparous and viviparous lizards and snakes (Fox 1977). The development and structure of corpora lutea found in Agama atra essentially showed the
typical reptilian pattern described by various workers. (Boyd 1940; Cunningham et al 1934; Weekes 1934; Altland 1951; Betz 1963; Goldberg 1970; Varma 1970; Veith 1974; Lance & Callard 1978; Cyrus et al 1978).

Once the ovum is expelled the follicle wall collapses and luteinization commences. The origin of the hypertrophied lutein cells is the granulosa layer, either by proliferation or differentiation as suggested by Weekes (1934) and Betz (1963). In the present study the lack of mitotic figures in the granulosa layer suggests that differentiation may be the more likely process. This corroborates suggestions by Betz (1963) and Varma (1970). Weekes (1934), however reported mitotic division in luteal cells of several lizards. Since immediate post-ovulatory follicles were not studied during the present investigation, no conclusion could be reached on the origin of the lutein cells. Although the thecal layer is thicker in the corpus luteum than in the preovulatory follicle no luteinization in the thecal layer could be detected in the present investigation. It is suggested that the increase in thecal thickness could have been the resulted shrinkage rather than cellular hypertrophy. Some workers however have reported the contrary (Weekes 1934; Altland 1951; Bragdon 1951). It is noteworthy that no distinct differentiation could be detected in the thecal layer of the preovulatory follicle in the female Agama atra, whereas in the corpus luteum distinct layers were observed. It remains questionable whether the two layers observed in the corpus luteum were comparable to the layers in the ripe follicle because no distinct difference in the composition of the two layers could be observed. Similar designations were made in the agamid lizard, Calotes versicolor (Varma 1970), the lizard, Sceloporus jarrovi (Goldberg 1970), snakes (Betz 1963; Lance & Callard 1978) and turtles (Altland 1951; Cyrus et al 1978). Boyd (1940) failed to recognise such corpus luteum thecal differentiation in the gecko, Hoplodactylus maculatus.
Most workers agree that the interior of the discharged follicle fills with hypertrophied granulosa cells (Fox 1977). Generally the lutein cells in the corpus luteum of Agama atra correspond to descriptions of hypertrophied lutein cells in the agamid lizard, Calotes (Varma 1970), the lizard, Sceloporus (Goldberg 1970) and the snake, Naja (Lance & Callard 1978) and Natrix (Betz 1963). Although the cytoplasm had a granulated appearance, the generally reported vacuolated cytoplasm was not observed in Agama atra. This discrepancy may be attributed to poor cytoplasmic staining ability.

The corpus luteum of most reptiles possess a less well-defined vascular system compared to the more intimate relation between luteal cells and blood vessels in mammals (Altland 1951). Moreover, the same author noted the absence of vascular tissue surrounding the luteal cells in the box turtle, Terrapene carolina. In most reptiles however, blood vessels were observed in at least the outer portion of the thecal layer (Fox 1977). In the corpus luteum of Agama atra extensive vascularization was evident throughout the thecal layer and erythrocytes were abundant in the luteal mass. Varna & Guraya (1973) also reported blood vessels in the lutein mass of Calotes versicolor. Vascularization of the lutein cells in oviparous reptiles is still doubtful and needs further investigation. In the present study it was found however, that shortly after the central cavity was filled with hypertrophied lutein cells, septal invasion of the lutein cells occurred. These septa from the theca interna were composed of collagenous fibres, fibroblasts and small blood vessels, thus resulting in a marked increase in corpus luteum vascularity. Similar observations were reported in the turtle (Cyrus et al 1978), snakes (Betz 1963; Lance & Callard 1978) and lizards (Goldberg 1970; Varma 1970). Samuel (1944, 1951) reported that the degree of connective tissue invasion and vascularity correlate with the degree of viviparity in snakes. Weekes (1934), however, did not reach the same conclusion for lizards. Panigel (1951) suggested that the degree of luteal vascularity is no measure of its physiological
activity. In Agama atra, however, it was clearly evident that the vascularity decreased in the degenerating corpus luteum and more information is needed before the view of Panigel (1951) can be supported.

In Agama atra a similar colloidal substance with a hyaline appearance, adjacent to the inner border of the theca and septa, similar to that found in the turtle, Chelydra serpentina (Cyrus et al 1978), was observed. Both Cyrus et al (1978) and Weekes (1934) reported this "coagulum" in association with corpus luteum degeneration. However, all stages of corpora lutea studied in Agama atra showed this substance reacting positive when stained with PAS. This neutral glycoprotein nature suggests the presence of a basal lamina, although a similar staining substance was noted in the degenerating corpus luteum and older remnants thus questioning the similarity of composition. The significance of the abundance of the colloidal substance in the remnants of corpora lutea is uncertain and Boyd (1940) suggested that it may be derived from the degeneration of collagen fibres.

In the present investigation the poor vascularization suggested a non-functional nature of the remnants in the female Agama atra. The increase in the number of pigment granules in the thecal layers may be responsible for the dark yellow coloration of the old (remnants) corpora lutea. The possible functional significance of the corpus luteum in the oviparous lizard have been discussed in paragraph 4.3.7.

3.4.3. The atresia of follicles
Several types of atretic follicles have been described for mammals (Ingram 1972), birds (Erpino 1973), fish (Reinboth 1972) and in the lizard, Calotes versicolor (Gouder et al 1979). However, in the majority of the lizard studies, only two types of atretic follicles have been described (Betz 1963; Varma 1970; Goldberg 1970; Eyeson 1971; Lance & Callard 1978). In accordance with the latter observations, only two types (hydration stage
and vitellogenic stage) of atretic follicles were distinguished during the annual reproductive cycle of *Agama atra*.

Several workers reported an increase in the number of atretic follicles in the postovulatory ovary (Tinkle 1961; Varma 1970; Goldberg 1970). Whilst Jones et al (1976) reported an increase in atresia during the previtellogenic and vitellogenic stages in *Anolis carolinensis*. These observations are consistent with results obtained in *Agama atra*, although occasional large vitellogenic stage atresia occurred. In ovaries where only a limited number of follicles reaches the preovulatory stage some method of selection must exist. Jones et al (1976) mentioned six possible selection processes. Whatever the selection process and its control, atresia will occur after every major selection process. Generally polyautochronic species have more than one germinal bed in order to produce adequate numbers of growing oocytes. In the present study however, only one germinal bed was observed in *Agama atra*. Varma (1970) also reported only one germinal bed for the agamid lizard, *Calotes versicolor*. One would therefore expect follicular atresia to be less prevalent in these species and it is evident that the degree of atresia may affect clutch size.

Hypophysectomy performed on *Anolis carolinensis* caused an increased in the number of follicular atresia, previtellogenic and vitellogenic follicles, whereas no increase occurred in small hydration stage follicular atresia (Jones et al 1976). Lance & Callard (1978) suggests that postovulatory atretic follicles may be caused by high progesterone levels, which is known to inhibit follicular development (Callard et al 1972a). In *Agama atra* however, large yolking follicles were observed in the postovulatory ovary in the presence of corpora lutea without an increase in atretic follicles. Therefore, the suggested function of progesterone as a follicular inhibitor would not be consistent with observations made in *Agama atra*. 
In the present investigation the onset of atresia marked by rapid hypertrophy of both granulosa and thecal layers and mitotic figures, which may be indicative of active hypertrophy, was frequently observed. Increased phagocytic activity was evident since the number of multilocular macrophages in the granulosa and ooplasm increased. There was also an increased vascularization and the abundance of mast cells in the hypertrophied thecal layer may be indicative of histamine vascular effects of oestradiol as suggested by Jones et al (1975b).

Barr (1968) considered atretic follicles to be degenerating elements of the ovary with doubtful endocrine function. Divergent and conflicting histochemical results have been reported up to date for non-mammalian atretic follicles. Gouder and his associates indicated that atretic follicles could be active sites of steroidogenesis in reptiles (Gouder et al 1976; Gouder et al 1979). Saidapur (1978) suggested that the histochemical visualization of certain enzymes may be remnants of pre-existing steroidogenic activity. Crews & Licht (1974) reported results that indicate that corpora atretica maintain sexual refractoriness by suppressing ovarian sensitivity to both environmental and hormonal stimulation in the lizard, Anolis carolinensis. From the above discussion it is apparent that the understanding of the endocrine role of the atretic follicle is inconclusive and it needs further study. Moreover, the possible correlation between plasma steroid levels and the histochemical activity of the atretic follicle needs to be investigated during the reproductive cycle of the oviparous lizard.

Several reports showed that in some lizards and snakes nuclei were present in the peripheral ooplasm of the developing previtellogenic follicles (Varma 1970; Guraya & Varma 1976; Jones & La Greek 1975a). They also reported that the nuclei disintegrated before yolk deposition starts, thus indicating that they are not remnants of pyriform cells. No such nuclei were however
observed in the ooplasm of developing previtellogenic follicles of *Agama atra*. Laughran et al. (1981) reported "rare" granular inclusion bodies of the peripheral ooplasm which may be similar to such nuclei reported by Guraya & Varma (1976) and Jones & La Greek (1975a). Laughran et al. (1981) suggested that such nuclei may originate from cells engulfed by young oocytes or alternatively may represent early evidence of atresia.

3.5. Conclusion

1. The germinal epithelium consists of a single elongated patch of cells on the dorsal surface of the ovary. The oogonia migrate into the ovarian stroma undergoing transformation into a primary oocyte, and become part of the follicular size hierarchy.

2. Oogonal proliferation, oogenesis and folliculogenesis in *Agama atra* appeared to be consistent with the generalization described by Tokarz (1979) in his extensive review.

3. Observations of the granulosa layer in growing follicles in the size hierarchy suggested that the large flask-shaped pyriform cells were derived from the intermediate cells which in turn were derived from the small basal cells. The functional significance of the cellular organization of the granulosa, especially the pyriform cells, remains obscure.

4. The role of the undifferentiated thecal layer appears to be primarily concerned with vascularization rather than steroid production, although further investigation is needed to support this suggestion.
5. The development and structure of the corpus luteum in *Agama atra* essentially showed the typical reptilian pattern. The functional significance of the corpus luteum especially during the onset of the second vitellogenic cycle needs further investigation.

6. Both hydration stage and vitellogenic stage atresia were recognised in *Agama atra*. The endocrine role of the atretic follicle is uncertain and the possible correlation between plasma steroid levels and histochemical activity of the atretic follicle needs investigation.
ABBREVIATIONS USED IN PHOTO-MICROGRAPHS

am = ameboid macrophage
ap = aperture in early corpus luteum
bc = basal cells
bm = basement membrane
bv = blood vessels
cv = connecting blood vessel
dpc = degenerating pyriform cell
e = erythrocytes
gc = granulocytes
GE = Germinal epithelium
Gr = Granulosa layer
gs = germinal stroma
H+E = Haematoxylin and eosin stain
H + PAS = Haematoxylin and Periodic acid stain
ic = intermediate cells
lm = lutein mass (granulosa)
lti = inner thecal layer of corpus luteum
lto = outer thecal layer of corpus luteum
mo = migrating oogonia
o = oogonia
op = ooplasm
os = ovarian stroma
ov = ooplasmic vacuoles
pc = pyriform cell
po = primary oocyte
sc = stromal cells
sm = static macrophage
tl = thecal layer
vm = vitelline membrane
zp = zona pellucida
zr = zona radiata
ys = yolk spheres
PLATE I

EXPLANATION OF FIGURES

13. Germinal epithelium of previtellogenic ovary (March) in Agama atra. Note the flattened periphery and the characteristic curl on one side (ventral). The ovarian stroma (os) shows a typical areolated appearance. o = oogonia; gs = germinal stroma; os = ovarian stroma; po = primary oocytes; H + E. (x 250).


15. Germinal epithelium (GE) with a primary oocyte (PO) surrounded by stroma cells (sc) adjacent to ovarian stroma (os) H + E (x 1000 oil).


17. Oil immersion micrograph of emerging primary oocyte. Note multilayered appearance of surrounding stromal cells. H + E (x 1000 oil).
PLATE II

EXPLANATION OF FIGURES

18. Germinal epithelium in preovulatory ovary. Note the limited amount of stromal tissue and the absence of primary oocytes in the GE. H + E (x 400).

19. Germinal epithelium in post-ovulatory III (January) ovary. Note the presence of migrating oogonia (mo) and a thicker GE H + E (x 400).

20. Primary oocyte in ovarian stroma (0.1 - 0.3 mm). Note the differentiation of the granulosa layer. H + PAS (x 400).

21. Oil immersion micrograph of differentiating granulosa layer of primary oocyte. H + PAS (x 1000).

22. Growing oocyte (0.3 - 0.5 mm) in ovarian stroma. Note the flattened anmesovarial side (arrow). H + PAS (x 400).

23. Cell differentiation in the granulosa layer of the growing oocyte (0.3 - 0.5 mm). Note the basal cells (bc) and the large intermediate cells (ic). Minute acidophilic inclusions (arrow) in ooplasm and visible H + PAS. (x 1000 oil).
PLATE III
PLATE III
EXPLANATION OF FIGURES

24. Thecal, granulosa and peripheral ooplasm of ovarian follicle (0.5 - 1.0 mm). Note pyriform cells (pc) and the distinct thecal layer (tl). H + PAS. (x 400).

25. Oil immersion micrograph of follicular wall. Note flask-shape pyriform cells (pc), intermediate cells (ic), small basal cells (bc), zone pellucida (zp) and vitelline membrane (vm) H + PAS (x 1000 oil).

26. Pyriform cell (pc) staining positive with PAS adjacent to unstained pyriform cells in granulosa layer (0.5 - 1.0 mm follicle). H + PAS (x 1000 oil).

27. High power view of figure 26 showing intercellular canal between pyriform cell (arrow) and oocyte. Note fibrous thecal layer. H + PAS (x 1000 oil).

28. Ovarian follicle (1 - 1.5 mm) showing extensive vacuolated appearance in ooplasm. Note acidophilic substance in the peripheral layers (arrow). H + PAS. (x 100).

29. Thecal, granulosa, zona pellucida, zona radiata and ooplasm of ovarian follicle (1 - 1.5 mm). Note acidophilic substance (arrow) and vacuoles (ov). H + PAS (x 400).
PLATE IV

EXPLANATION OF FIGURES

30. High power view of figure 29 showing the rounded pyriform cells and the radiated appearance of the zona radiata (zr). H + PAS (x 1000 oil).

31. Ovarian follicle (2.0 - 2.5 mm) collected in October. Note the thick zona pellucida (thick arrow) and basophilic yolk (arrow). H + PAS (x 250).

32. Ovarian follicle (2.0 - 2.5 mm) collected in March. Note the thick zona radiata, acidophilic substance and basophilic yolk granules (arrow). H + PAS. (x 400).

33. Ovarian follicle (2.5 - 3 mm). Note the presence of deutoplasmic yolk spheres, (arrow), the reduction in pyriform numbers and the thickened thecal layer. H + PAS. (x 400).

34. Oil immersion view of figure 33 showing two pyriform cells (pc), homogeneous zona pellucida (zp) and zona radiata (zr). Note the PAS positive staining substance (arrow) in the outer part of granulosa, dpc = degenerating pyriform cells. H + PAS. (x 1000).

35. Another high power view of figure 33 showing the signs of autolysis in the degeneration of the pyriform cells. Note the mast cells in the thecal layer (arrow). H + PAS. (x 1000 oil).
PLATE V
36. The active vitellogenic oocyte (3 - 4 mm). Note the cuboidal granulosa (Gr) the reduced zona pellucida (zp) and the vascularization (e) of the thecal H + PAS (x 400).

37. Oil immersion view of figure 36 showing the round, vesicular nuclei of the granulosa cells. H + PAS. (x 1000).

38. Preovulatory ovarian follicle (larger than 5 mm) showing the squamosal cells of the granulosa and large basophilic deutoplasmic yolk spheres (ys). H + PAS. (x 1000 oil).

39. Post-ovulatory ovarian follicle (corpus luteum) Stage I. Note the aperture (ap) and the hypertrophied granulosa (lutein) (lm) within the dark border (arrow) between lutein mass and thecal layer (tl). H + PAS (x 40).

40. Post-ovulatory follicle (corpus luteum). Note the three distinct layers, outer thecal layer (lto); inner thecal layer (lti) and granulosa lutein layer (lm); bloodvessels (bv) on border between outer theca and inner theca; cell "whorls" (arrow) near inner border of theca; basement membrane (bm) (small arrow) H + PAS (x 400).

41. Corpus luteum. Note bloodvessel on border between inner and outer border, perpendicular blood vessels (arrow) on border between theca and granulosa with a connecting vessel (cv) evident. H + PAS (x 400).
PLATE VI
PLATE VI

EXPLANATION OF FIGURES

42. Oil immersion micrograph of hypertrophied lutein cells. Note the vesicular nuclei with granular nucleoplasm. H + PAS (x 1000).

43. Septa of thecal connective tissue invade the lutein mass in mature (stage II) corpus luteum. Note large blood vessel (bv) and the colloidal substance (PAS positive) on the irregular border. H + PAS (x 400).

44. Lutein granulosa cells in a stage III corpus luteum. Note large intercellular spaces and smaller nuclei. H + PAS (x 1000 oil).

45. Degeneration of the corpus luteum. Note the irregular occurrence of the colloidal substance (PAS positive) (arrow). H + PAS (x 1000 oil).

46. Remnant of corpus luteum in the early vitellogenic ovary during the second vitellogenesis cycle: Note large quantities of colloidal (PAS positive) substance (arrow). H + PAS (x 10).

47. Hydration stage follicular atresia. Note the hypertrophied granulosa layer (Gr) and the connective tissue invasion from the thecal layer (ltl) (arrow). H + PAS (x 400).
48. Hydration stage atresia. Note the ameboid macrophage (am) in the ooplasm (op) and a static-macrophage (sm) attached to the granulosa (Gr). H + PAS (x 1000 oil).

49 & 50. Mitotic figures in the hypertrophied granulosa layer. H + PAS (x 1000 oil).

51. Mast cells (mc) were abundant in the thecal layer (tl). H + PAS (x 1000 oil).

52. Vitellogenic stage atresia. Note the fibrous theca (tl) hypertrophied granulosa (Gr) and signs (arrow) of vitelline membranes. Eroded yolk spheres (small arrows) are also evident. H + PAS (x 400).

53. Advanced vitellogenic atresia. Note hypertrophied granulosa (Gr) with strands of connective tissue penetrating the granulosa (arrow). Most of the yolk has been resorbed. H + PAS (x 400).
CHAPTER 4
THE PHYSIOLOGY OF VITELLOGENESIS IN THE FEMALE AGAMA ATRA

4.1. Introduction

Oocyte development in female oviparous lizards is usually associated with the accumulation of nutritive material within the cytoplasm, to be used during the early stages of embryonic development. These nutritive materials are often collectively referred to as "yolk", characterized by a high lipid and protein content. The process of yolk production is termed vitellogenesis and precedes ovulation and oviposition. The proportion of protein and lipid content as well as their total quantity in the cytoplasm varies among species. It is commonly assumed that the more highly evolved forms produce the most yolk-rich eggs (Pollet & Redshaw 1968). In this regard, it can be mentioned that the oocytes of marine echinoderm possess relatively little yolk which is mainly proteinaceous (oligolecithal egg) (Harvey 1965). The amphibian oocytes contain large quantities of yolk, normally divided unequally between the animal and vegetal poles (mesolecithal egg), but is not segregated completely from the cytoplasm surrounding the nucleus (Pollet & Redshaw 1968). The protein/lipid proportion also favours the proteinaceous content (Barth & Barth 1951). The oocytes of teleosts, reptiles and birds contain a large quantity of yolk, resulting in a complete segregation from the cytoplasm (telolecithal egg). As exemplified by the domestic fowl egg, the yolk contains relatively more lipid than protein. (Pollet & Redshaw 1974).

In order to produce these yolk materials drastic reorganization of metabolic activity precedes and accompanies vitellogenesis. Pronounced changes are therefore expected in vertebrate groups where a great amount of yolk is produced in a relatively short time. One of the most characteristic changes is the appearance of a soluble calcium binding lipophosphoprotein (vitellogenin).
acting as yolk precursor in the plasma. The presence of this complex in the plasma of a vitellogenic female lizard may therefore cause plasma levels of protein, calcium, lipids, phospholipids and phosphoproteins to be elevated above levels in non-vitellogenic females.

The present study was undertaken to investigate these expected plasma changes associated with vitellogenesis in the female *Agama atra*.

4.2. Procedure

4.2.1. Autopsy procedure
Described in paragraph 2.2.3.

4.2.2. Variation in plasma cholesterol
Total cholesterol was determined according to the micromethod of Mattenheimer (1971) using 10 µl of plasma. A cholesterol standard (400 mg. 100 ml⁻¹) was prepared from Cholesterol - (5)-01-(3β). (C₂₇H₄₆O). (Merck Chemicals). The absorbance values were determined with an Eppendorf 1101M photometer at 560 - 580 nm and the concentrations expressed as mg. 100 ml⁻¹.

4.2.3. Variation in plasma calcium
A primary standard of 1 000 mg. liter⁻¹ Ca⁺⁺ was prepared from which an aliquote was taken and diluted to 100 mg. liter⁻¹ Ca⁺⁺ with a diluent solution (0.03% NaCl; 0.5% LaCl and 5% T.C.A. in double distilled water). This solution was then used to prepare working standards ranging from 40 mg. liter⁻¹ Ca⁺⁺ to 1 mg. liter⁻¹ Ca⁺⁺ (figure 60). Plasma aliquots (100 µl) were diluted 100 times with a diluent solution (0.5% NaCl and 5% Trichloric acetic acid (TCA) in double distilled water). The samples were subsequently whirlimixed and centrifuged at 2 500 r.p.m. for ten minutes in a clinical centrifuge (Eppendorf) to remove the precipitated protein and the supernatant
was used for final determinations. Lanthanum chloride in both sample and standards served to reduce the interelementary interference. All glassware used in the calcium determinations were soaked overnight in 1N HCl and then rinsed in double distilled water and dried overnight at 250°C to avoid calcium contamination from residual organic material. The standard curve is presented in figure 60.

4.2.4. Variation in plasma protein

4.2.4.1. Total plasma protein concentration

Total plasma protein concentration was determined by the biuret reaction method (Henry et al 1957). A protein standard was prepared from lyophilized bovine serum (7.9 ± 0.1 gram protein 100 ml⁻¹) and/or from a standard solution (6g protein 100 ml⁻¹) purchased from Boehringer Mannheim. The absorbance values determined with an Eppendorf 1101M photometer at 530-580 nm, were used to calculate the protein concentration (mg. 100 ml⁻¹). When the assay mixture appeared turbid (lipemic serum), 150 ml diethyl ether was added, mixed for 30 seconds, centrifuged at 2 000 r.p.m. and the ether phase decanted, after freezing the aqueous phase.

4.2.4.2. Plasma protein fractions

Plasma protein fractions were separated on cellulose acetate membranes using a Beckman micro-zone electrophoresis system. A barbitol buffer (pH=8.6) was used and a constant voltage (250 volt) was applied for 20 minutes, whereafter the fractions were stained with a Ponceau S fixitive dye solution (Beckman). Following a rinse in acetic acid (5%) the membrane was dehydrated in denaturated alcohol. The background was subsequently cleared in a clearing solution (denaturated alcohol (70ml) and cyclohexanone (30ml). After drying (100°C) the zone density scanning was performed in a Beckman model R110 densitometer (slit length = 2.5 mm; slit width = 0.2 mm and a 520nm interference filter).
The optical density of each fraction was calculated from the integrator trace, and used as an estimation of the fraction concentration.

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.

4.2.5. **Seasonal variation in plasma progesterone**

The Radio-immunoassay (RIA) procedure used was described by Youssefnejadian et al (1972) and adapted by Faure (1975). In order to prevent steroid contamination all glassware was soaked in either Extran solution (Merck) or Lipsol solution (Lasec) and then thoroughly rinsed in tapwater followed by distilled water and finally absolute ethyl alcohol before dried at 250°C. All assays were standardised so that the same volumes were used throughout. The following solutions were prepared:

**Phosphate-buffered saline (P.B.S.)** contained 1% gelatin and 1% sodium azide. (pH = 7.0).

**Progesterone antiserum** raised in sheep was kindly provided by Dr J C Morgenthal (Department of Animal Physiology; Univ. of Stellenbosch, R.S.A.). The plasma was diluted (1 : 12 500) with P.B.S. and 0.2 ml used for each tube (Faure 1975).

**Tritiated progesterone** (Progesterone-1-H³. Radiochemical centre, Amersham) Stock solution (400 µl) was evaporated in a vacuum over (45°C). The residue was dissolved in a few drops of ethanol and made up to 100 ml with P.B.S. and 0.2 ml of this solution was used in each tube (20 nCi radioactivity).
Dextran-coated charcoal (1 gram activated charcoal (Merck) and 0.01 gram Dextran 150 suspended in 200 ml P.B.S. and stored at 4°C was used to separate bound and free progesterone.

4.2.5.1. Standard curve
Standards, ranging from 0.5 to 5 ng 0.2 ml⁻¹ were made up in P.B.S. Duplicate tubes containing buffer (P.B.S.) and standards were prepared, followed by the addition of 0.2 ml progesterone-anti serum(1/12500) and 0.2 ml tritiated progesterone (20 nCi radioactivity). The tubes were whirlimixed and incubated at 4°C overnight, whereafter 1 ml dextran-coated charcoal solution was added to separate bound and free progesterone. After mixing, the tubes were incubated for 15 minutes on ice and thereafter centrifuged for 5 minutes at 3 000 r.p.m. at 4°C. The supernatant, which contained the bound steroid was suspended in 10 ml scintillation cocktail (Insta-Gel; Packard) and counted in a Beckman LS-133 liquid scintillation counter to within an error of 1.5%. Counts per minute (CPM) obtained were calculated as a percentage of the CPM of the buffer blank (no steroid). The representing standard curve is presented in figure 64.

4.2.5.2. Extraction and progesterone determination
Plasma aliquots (0.1 - 0.2 ml) were made up to 0.5 ml with P.B.S. and 5 ml fresh, distilled ether added to each tube. After mixing for 30 seconds, the ether phase was separated from the plasma by quick freezing (dry ice) the lower aqueous phase and decanting ether phase into a clean tube. The ether was then evaporated under a stream of air with the tubes standing in a water-bath (45°C). To the dried extract 0.2 ml P.B.S. was added in order to obtain the same reagent volume as the standards. The buffer blank and female plasma aliquots were treated identically to assay procedure for standards (see above).
Accuracy of the assay was determined by extracting a standard sample (3 ng ml\(^{-1}\)) simultaneously with plasma aliquots and the measured concentration calculated as a percentage of the expected concentrations (Extraction yield). Before any plasma samples were assayed a series of assays were performed until the same extraction yield was obtained for each assay. From this it was found that an overestimation of 12% could be predicted for each assay.

4.3. Results and discussion

4.3.1. Seasonal variation in fatbody index

In most reptiles, the bulk of the lipids are stored subcutaneously or in visceral fatbodies (corpora adiposa). The fatbody index (wet fatbody mass \(\frac{100 \text{ g body mass}^{-1}}{100 \text{ g body mass}^{-1}}\)) is a widely used indicator of lipid cycling patterns (Derickson 1974, 1976) although not indicative of the absolute quantity of storage lipids available. Moreover, Derickson (1974) demonstrated that fatbody lipids are the most labile, thus storage or utilization of lipids would be apparent in this depot first.

In the female *Agama atra* most of the lipids were stored in paired abdominal - situated corpora adiposa, although occasional subcutaneous fatbodies were present in the pectoral region. The seasonal variation in the fatbody index is presented in figure 54. Large fatbodies occurred during the winter months, coinciding with a typical quiescent ovarian condition. (3 - 5g 100g\(^{-1}\)). The onset of the breeding season was marked by a reduction in the fatbody index. (\(p<0.02\), April vs August). The reduction of the fatbody index continued during deutoplasmic activity in the ovarian follicles until after ovulation occurred (figure 54) (\(p<0.001\), April vs Post-ovulatory I). However non-significant, an increase in fatbody index occurred in females containing oviducal eggs (0.1\(p>0.05\); Post I vs Post II). The onset of a second
Figure 54.

Variation in the fatbody index during the reproductive cycle of the female *Agama atra*. Each point represents the mean ± standard deviation (SD) for each stage. The sample size is given above the bars.
vitellogenesis cycle was marked by a reduction in fatbody mass, reaching a seasonal minimum before oviposition of a second clutch of eggs. (P<0.02; Post II vs Pre II). A dramatic increase followed in February to reach a seasonal maximum in April (P<0.001; Post III vs April).

Four patterns of lipid storage and utilization in lizards have been reported. Firstly, tropical species show no lipid cycling and apparently undergo no seasonal cycle of reproductive activity. (Church 1962; Hoddenbach & Lannom 1976; Licht & Gorman 1970; Derickson 1976). Secondly, some lizard species may utilize their lipids primarily for maintenance during winter dormancy. - (Dessauer 1955). Thirdly, a number of studies indicate that some lizards store lipids during the breeding season. (Hahn & Tinkle 1965; Hoddenbach 1966; Smith 1968; Minnich 1971; Licht & Gorman 1970). Lastly, several studies have indicated that some lizard species utilize their lipids for maintenance during dormancy and reproduction. (Telford 1970; Goldberg 1970; Gaffney & Fitzpatrick 1973).

The inverse relationship between the fatbody cycle and ovarian cycle in the female *Agama atra*, the iguanid lizard, *Sceloporus jarrovi* (Goldberg 1970) and the cobra, *Naja naja* (Lance 1975) furthermore suggest that lipids appear to be used predominantly for reproduction. Dessauer's study (1955) is the only one that clearly indicated the use of lipids exclusively for maintenance during dormancy.

Hahn & Tinkle (1965) mentioned the possibility of lipid deposition between successive ovulation cycles in the lizard, *Uta stansburiana*. The fatbody cycle of *Agama atra* clearly showed two lipid storage cycles which coincided with quiescent, ovarian conditions. Chapman & Chapman (1964) reported similar
observations for Agama agama. The slight increase in fatbody mass between successive vitellogenesis cycles in Agama atra corroborate the suggestion that lipid storage was primarily utilized for reproduction.

Experimental studies have demonstrated the importance of fatbodies for the completion of vitellogenesis (Hahn & Tinkle 1965; Tinkle 1965; Burrage 1973). Fatbody excision before the onset of vitellogenesis inhibited ovarian development whereas removal during vitellogenesis resulted in an increased occurrence of follicular atresia. Burrage (1973) reported terminations in pregnancy, abortion or resorption or early stage embryos after excision experiments in the viviparous chameleon, C. pumilus. However, no pregnancy terminations occurred in females containing later stage embryos. He therefore concluded that the fatbodies in C. pumilus may have a dual role, namely lipid mobilization for follicular development and sustenance of later stage embryos. In the light of his report it seems more likely that the fatbodies were necessary for the sustenance of the earlier embryos than the later stage embryos. Nevertheless, the excision result in rapid fatbody lipids and egg lipids, strongly suggest that fatbody lipids are primarily used for follicular development (Hahn & Tinkle 1965; Burrage 1973).

Dessauer (1955) in his comprehensive study on the seasonal changes in distribution of proteins, carbohydrates and lipids in various tissues of the iguanid lizard, Anolis carolinensis, reported a marked turnover and exchange of lipid between fatbody and liver. Lance (1975) suggested the same functional relationship between liver and fatbody in the cobra, Naja naja. According to Hahn (1967) the liver is probably an intermediary organ for both the storage and utilization of lipids. Lance (1975) suggested that plasma
lipids were first mobilized into the fatbodies before being passed on to the liver. Afroz et al (1971) made similar suggestions. Although no significant change could be detected in the hepatosomatic index in the female Agama atra, a slight decrease in liver mass before the deposition of the fatbodies suggested a relationship with the liver (figures 54 and 63). A similar inverse relationship before the onset of vitellogenesis may further corroborate the fatbody liver interrelationship (figures 54 and 63).

Tinkle (1965) postulated that a female containing large fatbodies at the onset of vitellogenesis may be better prepared for the production of the first clutch thereby contributing to the number of offspring surviving to adulthood. In most lizards, however, the fatbodies remain reduced throughout the subsequent ovulation cycles which may suggest that the fatbody itself is not essential for reproduction. Derickson (1976) suggested that food availability is the ultimate factor determining whether or not an organism needs to store lipids. If food levels fluctuate seasonally it is likely that lipids will be stored. Furthermore, seasonal fluctuations in food availability could be the result of seasonal fluctuations in precipitation and environmental temperatures. In the temperate zone, photothermal factors may influence the insect production, resulting in a decrease during the winter months. Derickson (1976) made a similar suggestion for Agama agama. Therefore, in order to meet metabolic demands during the onset of vitellogenesis, lizards at temperate latitudes may store lipids prior to winter months and utilize these stores partly during these months with the remaining lipids being used for reproduction. The diet of Agama atra being mainly insectivorous corroborates the above suggestion. However, figure 54 indicates that the fatbody index progressively declines throughout the winter months, thus suggesting lipid utilization for metabolic demands (April vs July). During the summer months (breeding stages) adequate food availability would make large fatbodies unnecessary. Goldberg & Robinson (1979) reported on two Namib desert lacertids which showed fatbody cycles typical of temperate lizards, although only one, Meroles canurostris,
exhibited a temperate reproduction cycle. The other lizard, *Aporosaura anchiaetae*, was reproductively active throughout the year. They therefore concluded that nutritional factors may be the primary regulators of reproductive cycles in the desert ecosystem.

Hahn & Tinkle (1965) suggested an endocrine basis for the regulation of the fatbody cycle in temperate zone lizards. Ovariectomy studies suggested that the fatbody may be a target organ for oestrogen. However, the onset of vitellogenesis is characterized by activity in a number of endocrine organs (pituitary, adrenal, etc) which could influence the fatbody.

From the available information it is evident that more research is needed to elucidate the functional significance of the fatbodies in temperate zone lizards.

4.3.2. Seasonal variations in plasma cholesterol

Increased plasma cholesterol levels during the breeding season have been reported for a number of vertebrates. These include teleosts (Idler & Tsuyuki 1958; Lewander et al 1968) and birds, (Hoffman 1960; Sturkie 1965; Kern et al 1972), but there is little information on the relationship between reproduction and plasma cholesterol levels in reptiles.

The seasonal variation in plasma cholesterol in *Agama atra* is presented in figure 55. The highest cholesterol values were recorded in autumn, (March; 334 ± 37mg 100⁻¹) coinciding with the maximum fatbody index. A marked decrease occurred during the late previtellogenic stages (P<0.001; May vs July) coinciding with a reduced fatbody index. Cholesterol levels remained relatively low during vitellogenesis (August - Pre I) although a significant increase was recorded during the second vitellogenic stage (P<0.002; Post-
Figure 55.
Variation in the total plasma cholesterol concentration during the reproductive cycle of the female Agama atra. Symbols as in fig. 54.
<table>
<thead>
<tr>
<th>ORDER</th>
<th>FAMILY</th>
<th>SPECIES</th>
<th>n</th>
<th>x ± SD</th>
<th>RANGE</th>
<th>REFERENCES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crocodilia</td>
<td>Crocodylidae</td>
<td>Alligator mississippi</td>
<td>157</td>
<td>50</td>
<td>22 - 88</td>
<td>Coulson &amp; Hernandez 1964</td>
</tr>
<tr>
<td></td>
<td></td>
<td>piensis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chelonia</td>
<td>Chelididae</td>
<td>Clemmys insculpta</td>
<td>3</td>
<td>343 ± 47</td>
<td></td>
<td>Stenroos &amp; Bowman 1968</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sternothaerus odoratus</td>
<td>8</td>
<td>376 ± 32</td>
<td>260 - 570</td>
<td>Jackson et al 1971</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sternothaerus minor</td>
<td>15</td>
<td>506 ± 23</td>
<td>360 - 670</td>
<td>Jackson et al 1971</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Testudinida carolina minor</td>
<td>31</td>
<td>340 ± 16</td>
<td>178 - 511</td>
<td>Hollomb et al 1972</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chrysemys picta belli</td>
<td>51</td>
<td>138 ± 33</td>
<td></td>
<td>Chaikoff &amp; Enterman 1946</td>
</tr>
<tr>
<td></td>
<td>Testudinida</td>
<td>Chrysemys picta belli</td>
<td>6</td>
<td>261 ± 26</td>
<td></td>
<td>Stenroos &amp; Bowman 1968</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chrysemys scripta</td>
<td>8</td>
<td>290 ± 42</td>
<td>174 + 512</td>
<td>Colcomb et al 1972</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chrysemys scripta elegans</td>
<td>2</td>
<td>69 ± 3</td>
<td></td>
<td>Stenroos &amp; Bowman 1968</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chrysemys floridana peninsularis</td>
<td>12</td>
<td>304 ± 12</td>
<td>255 - 362</td>
<td>Colcomb et al 1972</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pseudodocima blondingi</td>
<td>5</td>
<td>253 ± 31</td>
<td></td>
<td>Stenroos &amp; Bowman 1968</td>
</tr>
<tr>
<td>Squamata</td>
<td>Varanidae</td>
<td>Varanus niloticus</td>
<td>1</td>
<td>152</td>
<td></td>
<td>Vastesaeger et al 1965</td>
</tr>
<tr>
<td>Agamidae</td>
<td></td>
<td>Uromastic harwickii</td>
<td>10</td>
<td>162 ± 9</td>
<td>130 - 212</td>
<td>Rangneker &amp; Suryavanshi 1969</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Agama atra</td>
<td>5 - 8</td>
<td>200 ± 334</td>
<td>220 - 334</td>
<td>Present study</td>
</tr>
<tr>
<td>Agamidae</td>
<td></td>
<td>Xenochrophis piscator</td>
<td>15</td>
<td>227 ± 18</td>
<td></td>
<td>Rangneker &amp; Padgaonbar 1972</td>
</tr>
<tr>
<td>Elapidae</td>
<td>Naja naja</td>
<td>10 - 28</td>
<td>200 - 300</td>
<td>155 - 550</td>
<td>Lance 1975</td>
<td></td>
</tr>
<tr>
<td>Viperidae</td>
<td>Erotalus atrox</td>
<td>12</td>
<td>158 ± 46</td>
<td>75 - 235</td>
<td>Martin &amp; Bagby 1973</td>
<td></td>
</tr>
</tbody>
</table>
ovulatory II). The second pre-ovulatory stage was marked by decreased cholesterol levels which remained low until April (P<0.002; Post-ovulatory II vs Pre-ovulatory II). Table 1 summarizes information on the blood cholesterol levels measured in other reptiles. In most cases neither time of the year nor the sex is given.

Dessauer (1955) reported a marked turnover and exchange of lipids between the liver and the fatbody. Afroz et al (1971) showed that the fatbody of the lizard, Uromastix harwickii, contained a high proportion of cholesterol compared to mammalian deposition whereas the cholesterol content declined during fatbody regression. Lance (1975) explained the drop in plasma cholesterol prior to fatbody deposition as the result of fatbody uptake in the female cobra, Naja naja. In the present investigation similar observations were recorded during fatbody deposition in Agama atra. On the other hand, the increase in cholesterol during August may be the result of decreased fatbody uptake. Lance (1975) suggested a strong negative correlation between ovarian mass and plasma cholesterol in the cobra, Naja naja. A similar decrease in plasma cholesterol in Agama atra suggested a possible correlation with ovarian maturation (July – Pos I). Increased plasma cholesterol levels during the second vitellogenic stage in Agama atra may be ascribed to increased cholesterol synthesis in the liver followed by a subsequent decrease during the preovulatory stage. However, the phenomenon is generally observed in birds where plasma cholesterol increases during vitellogenesis and egg laying (Romanoff & Romanoff 1949; Mukherjee et al 1969).

Cholesterol may be incorporated into low density lipoproteins which may account for 95% of yolk lipids (Martin & Bagby 1973). The lipid content of ovarian follicles increased during follicular growth in the turtle, Clemmys insculpta (Brenner 1970). Similar accumulation of lipid and cholesterol in the developing follicle has been reported in teleosts (Lewander et al 1974). Fernholm (1972) reported a possible cholesterol accumulation in the
eggs of *Myxine glutinosa* (Cyclostomata) after treatment with labelled cholesterol. Similar reports came from Romanoff & Romanoff (1949), who studied the egg of the domestic fowl. The evidence therefore corroborates the hypothesis that lipid (cholesterol) may be selectively accumulated by the developing follicle and in this regard Wiegand & Peter (1980) suggested that the major portion of ovarian cholesterol comes from exogenous sources via the plasma. When the ovarian uptake of plasma cholesterol exceeds the output from the source(s) as follicular growth proceeds, a decline in plasma levels can be expected. Oestrogens have been reported to increase the synthesis and subsequent release into the plasma of various plasma lipids in most non-mammalian vertebrates, (Wiegand & Peter 1980; Pollet & Redshaw 1968, 1974; Fox 1977). Pollet & Redshaw (1968) suggested that gonadotropin treatment caused an increased uptake of labelled vitellogenin and circulating esterified cholesterol by amphibian ovarian follicles, and Van Tienhoven (1968) reported that gonadotropins increased the permeability of the egg membranes. It may therefore be suggested that a drop in plasma cholesterol during vitellogenesis could be due to increased plasma gonadotropins resulting in a rapid cholesterol uptake by the ovary. It still remains to be determined if such a vitellogenesis surge in gonadotropin release exists in the female *Agama atra*.

The control of cholesterol metabolism seems to be extremely complex. Several factors such as age, sex, diet, genetic constitution and a number of hormones have been implicated in its regulation (Ho & Taylor 1970). Further research is needed in order to elucidate the role of cholesterol in the reproduction of non-mammalian vertebrates.

4.3.3. Seasonal variations in plasma proteins

Three to seven percent of lizard blood plasma is comprised of a complex mixture of proteins, with different structure and properties (Dessauer 1970).
Several factors such as stage of development, sex, season and physiological stage may effect the protein compliment of the plasma. In females of oviparous vertebrates the period preceding oviposition in invariably marked by a phase of vitellogenesis. The presence of the soluble yolk precursor (Calcium-binding lipophosphoprotein = vitellogenin) in the plasma of vitellogenic females may cause characteristic seasonal changes in the plasma protein compliment.

The plasma proteins of the female *Agama atra* resolved into five major fractions upon cellulose acetate electrophoresis in alkaline buffers (figure 56). Human plasma protein fractions were used as reference standards of protein mobilities during electrophoresis.

Fraction I showed very little mobility towards the cathode electrode and correspond to the migration distance of the gamma-globulins present in human plasma. The mobility of fraction II correlated with that of the human beta-globulins and fraction III corresponded to the mobility of the alpha-globulin fraction in human plasma. Likewise, the mobility of fraction IV corresponded to that of the human albumins, but the mobility of fraction V exceeded the migration rate of human albumins.

The seasonal variation in the total plasma protein concentration and that of the individual plasma protein fractions are presented in figures 57, 58 and 59 and tables 2 and 3.

Concomitant with a minimum gonadosomatic index during the winter months (figure 9) the total plasma protein concentration reached its maximum plasma level ($4.32 \pm 0.59g.100ml^{-1}$) during April (figure 57). The onset of vitellogenesis was marked by significantly decreased total plasma proteins ($P<0.01$; April vs August) followed by a slight rise during the first
The separation of the plasma protein fractions upon cellulose acetate electrophoresis. Human plasma proteins (top), non-vitellogenic female Agama atra (middle) and vitellogetic female Agama atra (bottom). alb = albumin; a = alpha-globulins; $\gamma$ = gamma-globulins, and $\theta$ = fibrinogen. The numbering of the reptilian fractions are for convenience. Arrows indicates applications point.
preovulatory stage (P<0.1 August vs Pre I). A significant rise was evident during the second vitellogenic stage (P<0.0001: Post I vs Post II), whereas a reduction followed during the subsequent postovulatory stages.

Fraction V reached higher concentration levels than any other fractions and made the largest single contribution to the total protein compliment (39 - 41% winter months) (figure 59). The foremost migrating fractions (V, IV, III) showed the same seasonal pattern of variations, namely reduced concentration during the vitellogenic stages. The slower migrating fraction II did not change much outside the breeding season, however, during vitellogenic activity a marked increased was evident. Although Fraction I showed considerable variations throughout the reproductive cycle, increased levels were evident during the second vitellogenic cycle (figure 58). The ratio, fraction II : fraction V clearly showed increased concentration of fraction II during peak vitellogenic period (figure 59).

The total plasma protein concentration (T.P.P.) is the female Agama atra showed considerable individual variation. One uncontrolled variable was the period of time between capture in the field and arrival in the laboratory. However, it was ensured that the collected lizards were autopsied within 48 hours after capture. Furthermore, all the lizards examined had full stomachs and appeared to be in good condition.

Generally an inverse correlation between total plasma protein concentration and gonadosomatic index was recorded (r = 0.77), but during the second vitellogenic cycle an increase in T.P.P. was recorded. (P<0.001; Post I vs Post II). Callard et al (1978) recorded no significant changes in the total protein concentration during the annual ovarian cycle of the turtle, Chrysemys picta. Their results are in contrast to those reported by Clark (1967) for the same species, namely an increase in T.P.P. during
Figure 57.

Variation in the total plasma protein concentration during the reproductive cycle of the female Agama atra. Symbols as in fig. 54.
<table>
<thead>
<tr>
<th>Reproductive Stages</th>
<th>Total Protein (g.100 ml⁻¹)</th>
<th>Plasma Protein Fractions (% of Total Proteins)</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>APRIL</td>
<td>4.32 ± 0.59</td>
<td></td>
<td>5.43 ± 0.98</td>
<td>19.14 ± 4.34</td>
<td>23.86 ± 3.76</td>
<td>12.86 ± 1.96</td>
<td>39.40 ± 1.51</td>
</tr>
<tr>
<td>MAY</td>
<td>4.16 ± 0.93</td>
<td></td>
<td>6.80 ± 1.48</td>
<td>23.00 ± 2.24</td>
<td>19.80 ± 1.64</td>
<td>10.60 ± 2.30</td>
<td>39.40 ± 1.30</td>
</tr>
<tr>
<td>JULY</td>
<td>3.78 ± 0.54</td>
<td></td>
<td>8.33 ± 1.53</td>
<td>21.33 ± 1.53</td>
<td>20.33 ± 1.53</td>
<td>8.67 ± 1.52</td>
<td>41.33 ± 2.52</td>
</tr>
<tr>
<td>AUGUST</td>
<td>3.13 ± 0.80</td>
<td></td>
<td>6.67 ± 2.08</td>
<td>28.67 ± 2.31</td>
<td>19.33 ± 5.86</td>
<td>7.33 ± 0.58</td>
<td>38.00 ± 2.65</td>
</tr>
<tr>
<td>PRE I</td>
<td>3.40 ± 0.90</td>
<td></td>
<td>7.50 ± 0.84</td>
<td>32.83 ± 3.19</td>
<td>18.67 ± 3.98</td>
<td>8.17 ± 2.48</td>
<td>33.34 ± 3.08</td>
</tr>
<tr>
<td>POST I</td>
<td>2.74 ± 0.40</td>
<td></td>
<td>8.50 ± 2.24</td>
<td>24.00 ± 0.89</td>
<td>21.33 ± 3.67</td>
<td>8.67 ± 1.51</td>
<td>37.67 ± 2.73</td>
</tr>
<tr>
<td>POST II</td>
<td>4.08 ± 0.50</td>
<td></td>
<td>8.50 ± 1.64</td>
<td>31.61 ± 2.73</td>
<td>21.14 ± 2.14</td>
<td>10.50 ± 1.87</td>
<td>28.25 ± 1.94</td>
</tr>
<tr>
<td>PRE II</td>
<td>3.36 ± 0.60</td>
<td></td>
<td>7.67 ± 2.08</td>
<td>29.67 ± 1.14</td>
<td>19.33 ± 4.73</td>
<td>8.00 ± 2.00</td>
<td>35.33 ± 2.08</td>
</tr>
<tr>
<td>POST III</td>
<td>3.21 ± 0.77</td>
<td></td>
<td>6.60 ± 2.51</td>
<td>22.20 ± 1.64</td>
<td>22.00 ± 1.58</td>
<td>9.60 ± 1.10</td>
<td>39.40 ± 1.52</td>
</tr>
<tr>
<td>FEBR.</td>
<td>3.75 ± 0.56</td>
<td></td>
<td>8.50 ± 1.73</td>
<td>19.00 ± 1.41</td>
<td>22.75 ± 0.96</td>
<td>11.00 ± 1.41</td>
<td>38.75 ± 2.36</td>
</tr>
</tbody>
</table>
TABLE 3: VARIATION IN QUANTITATIVE CONCENTRATION OF PROTEIN FRACTIONS TO TOTAL PROTEIN (MEAN ± SD)x

<table>
<thead>
<tr>
<th>n</th>
<th>Reproductive Stages</th>
<th>Total Protein g.100 ml⁻¹</th>
<th>PLASMA PROTEIN FRACTIONS (QUANTITATIVE CONCENTRATION) g.100ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>7</td>
<td>APRIL</td>
<td>4.32 ± 0.59</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>6</td>
<td>MAY</td>
<td>4.16 ± 0.93</td>
<td>0.28 ± 0.06</td>
</tr>
<tr>
<td>5</td>
<td>JULY</td>
<td>3.78 ± 0.54</td>
<td>0.32 ± 0.06</td>
</tr>
<tr>
<td>6</td>
<td>AUGUST</td>
<td>3.13 ± 0.80</td>
<td>0.21 ± 0.07</td>
</tr>
<tr>
<td>8</td>
<td>PRE I</td>
<td>3.40 ± 0.90</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>POST I</td>
<td>2.74 ± 0.47</td>
<td>0.23 ± 0.06</td>
</tr>
<tr>
<td>6</td>
<td>POST II</td>
<td>4.08 ± 0.80</td>
<td>0.35 ± 0.07</td>
</tr>
<tr>
<td>5</td>
<td>PRE II</td>
<td>3.36 ± 0.06</td>
<td>0.26 ± 0.03</td>
</tr>
<tr>
<td>5</td>
<td>PRE III</td>
<td>3.21 ± 0.77</td>
<td>0.21 ± 0.08</td>
</tr>
<tr>
<td>6</td>
<td>FEBRUARY</td>
<td>3.75 ± 0.56</td>
<td>0.32 ± 0.06</td>
</tr>
</tbody>
</table>

x = Student t-test probability (P).
Values with similar codes were compared.

ₐ = P < 0.001. Highly significantly different.
Figure 58.
Variation in the plasma protein fractions concentrations as measured by cellulose-acetate electrophoresis: Fractions I, II and III.
Figure 59. Variation in the plasma protein fraction concentrations as measured by cellulose-acetate electrophoresis: Fractions IV, V and the ratio II:V
the vitellogenic stages. Dessauer et al (1956) and Dessauer & Fox (1958) studied the changes in plasma proteins during the reproductive cycles of fourteen forms of gartersnakes, Thamnophis and ten forms of watersnakes, Natrix. Their findings indicated that the total plasma protein concentration reached a peak just before ovulation, followed by a decrease during early pregnancy. Pollet & Redshaw (1968) published results indicating that implantations of oestradiol - 17β in the female Xenopus laevis caused a threefold increase in total plasma protein concentration. Similar increases were reported in birds (Deely et al 1975; Bergink et al 1974) and teleosts (Le Menn 1979) whereas no significant changes were recorded in the oviparous elasmobranch, Scyliorhinus canivula. (Craik 1978).

Seasonal changes in the plasma concentrations of the individual plasma protein fractions may disguise significant trends in the total plasma concentration. Callard et al (1978) and Gapp et al (1979)suggested a decrease in hepatic synthesis of "other" proteins concomitant with increased vitellogenin concentration in the plasma, resulting in no significant changes in the T.P.P. in the turtle, Chrysemys picta. A similar explanation was used for the relatively small seasonal variation in T.P.P. recorded in the lizards, Dipsosaurus dorsalis (Gerstle & Callard 1972) and Uta stansburiana (Hahn 1967). A similar phenomenon was observed in Agama atra, but, changes in T.P.P. were still evident. It has also been suggested that the increase in foremost migrating fractions (especially, "albumin") may indicate a possible regulation of plasma osmolarity during vitellogenesis (Hahn 1967; Gapp et al 1979).

Generally the process of yolk deposition in the oocyte is preceded by the emergence of a "new" plasma protein component. In amphibians the yolk protein precursor has been called serum lipophosphoprotein (S.L.P.P.) (Wallace & Jared 1968) Xenoprotein (Munday et al 1968) and vitellogenin
In reptiles the precursor was often called, a serum vitellin (Hahn 1967) or phopholipoprotein (P.L.P.) complex (Gerstle & Callard 1972). Pan et al (1969) proposed that the generic term vitellogenin include all macro-molecule yolkprotein precursors in the hemolymph of certain insects. Since then the term has been adopted widely, although purely in a fuctional capacity. This does not necessarily mean that insect vitellogenin is the same protein as found in vertebrates.

Plasma collected from vitellogenic Agama atra females was characterized by a significant increase in fraction II (P<0.001; April vs Pre-ovulatory I) (P<0.001; Postovulatory I vs Postovulatory II). The relatively high levels of fraction II in the plasma throughout the year indicated that this protein fraction was not confined to the period of vitellogenesis. It is assumed that incomplete separation of plasma protein components occurred in view of the relatively low resolution achieved with cellulose acetate electrophoresis.

Undenatured plasma vitellogenin of the lizards, Anolis carolinensis (Rosenquist, 1969 cf. Dessauer 1974) and Dipsosaurus dorsalis (Gerstle & Callard 1972); numerous snakes, Thamnophis and Natrix (Dessauer & Fox 1958, 1959); the alligator, Alligator mississippiensis (Van Brunt et al 1971 cf. Dessauer 1974) and turtles (Dessauer 1964), migrated in the β region when subjected to electrophoresis. Suzuki et al (1968) recorded a pronounced increase in the "γ-globulin" electrophoretic component in the lizard, Sceloporus cyanogenys. Similar elevations in the γ-globulin fractions were reported in Chamaeleo pumilus pumilus following 17β-oestradiol therapy (Veith 1974). However, no significant changes could be detected during the natural reproductive cycle.
In the literature it is generally suggested that vitellogenin is a high molecular weight protein. In the amphibian, *Xenopus laevis*, the molecular weight of the native vitellogenin molecule is reported to be 460,000 daltons (Wallace 1979) and in the chicken 480,000 daltons (Deeley et al 1975). Furthermore, under denaturing conditions on SDS-polyacrylamide gels the turtle, *Chrysemys picta*, has one polypeptide subunit of 210,000 - 220,000 daltons, being similar to chicken vitellogenin subunits (220,000 daltons) (Deeley et al 1975) and larger than *Xenopus* vitellogenin subunits (195,000 daltons) (Bergink et al 1974; Ho et al 1980) suggested that turtle vitellogenin is also composed of two polypeptides of equal size (210,000 daltons). However, the electrophoretic form is composed only of the 210,000 dalton form. This is in contrast to teleost and avian vitellogenin which appear to exist in several electrophoretically separable forms (serum phosphovitin and lipovitellin) (Wallace 1978).

The new plasma protein fraction (vitellogenin) was found to be immunologically similar to the major oocyte yolk proteins in the domestic fowl (reviewed by Schjeide et al 1963). Neaves (1972) has demonstrated that tracer substances pass through the extracellular spaces of the follicular epithelium and are incorporated into the developing oocyte by micropinocytosis in the lizard, *Anolis carolinensis*. Similar observations were made in the growing oocyte of the turtle, *Pseudemys scripta elegans* (Rahil & Narbaitz 1973). The uptake of vitellogenin by the oocyte appears to be a highly selective process, resulting in a more rapid uptake than other proteins (Wallace & Bergink 1974). It is suggested that gonadotropins may in some way stimulate the process of micropinocytosis in *Xenopus*, and possibly also in other species, resulting in the entrance of such large macromolecules into the oocyte (Follet & Redshaw 1978).
The enormous concentration of protein found in the oocyte suggests a large differential which could lead to a concentration gradient between the oocyte and plasma with regard to vitellogenin. However, soluble yolk precursor with its high osmotic potential is rapidly immobilized to an insoluble nonlabile form in the oocyte.

Vitellogenin has never been isolated as such from the oocyte, but found in yolk platelets as crystalline structures, containing almost exclusively the proteins, lipovitellin and phosvitin (Wallace & Bergink 1974). Data presented by Ho et al (1980) suggested that turtle yolk is relatively simple in its composition, consisting of one phosvitin and possibly one lipovitellin (composed of two subunits). The yolk of *Xenopus laevis* consists only of one phosvitin and one lipovitellin (Wallace 1965). The egg yolk of the domestic fowl shows a greater complexity containing phosvitin, $\beta$-lipovitellins and $x-\beta$ and $x$-livetins (Wallace 1978).

4.3.4. Variation in total plasma calcium

Variation in total plasma calcium concentration in the female *Agama atra* is presented in figure 61.

Outside the breeding months, the total calcium concentration showed little variation with values ranging between 112 - 132 mg.100ml$^{-1}$ (February - July). Despite considerable individual variation the mean total plasma calcium showed a highly significant increase during the first vitellogenic period ($P<0.001$, July vs Preovulatory I). A decrease in total calcium concentration was recorded in the presence of small hydration stage ovarian follicles and oviducal eggs. A highly significant increase in calcium levels followed concurrently with an increase in vitellogenic activities during oviposition of the first clutch ($P<0.001$ Postovulatory I vs Post-ovulatory II). The highest seasonal plasma calcium concentration was
Figure 60.
Standard concentration curve used to determine total plasma calcium. \( r = 0.9977 \)
Figure 61.

Variation in the total plasma calcium concentration during the reproductive cycle of the female Agama atra. Symbols as in fig. 54.
recorded during the second vitellogenic phase \((270 \pm 37.33 \text{ mg.100ml}^{-1})\). Females containing large preovulatory ovarian follicles (second clutch) showed relatively low total calcium levels \((116 \pm 70.38 \text{ mg.100ml}^{-1})\), \((P<0.002; \text{ Postovulatory II vs Preovulatory II})\). After a continuous decrease, the total calcium concentration reach its lowest value during the second oviposition period \((87.54 \pm 20.00\text{mg.100ml}^{-1})\) \((P<0.002; \text{ February vs March})\).

Dessauer et al (1956) and Dessauer & Fox (1958) measured calcium concentrations in two genera of ovo-viviparous snakes, Thamnophis and Natrix during the reproductive cycle and reported a dramatic increase during vitellogenesis. The increase was found to be as high as thirty times the normal level in the snake plasma during the time of ovulation. Shortly after ovulation the calcium content of the plasma returned to normal. Plasma calcium concentration increased significantly during the period of maximum ovarian follicular development in the cobra, Naja naja (Lance 1975). Increased pre-ovulatory calcium concentration was also recorded in the lizards, Dipsosaurus dorsalis (Gerstle et al 1972) and Sceloporus cyanogenys (Suzuki & Prosser 1968).

In the present investigation the highest recorded mean value in the female Agama atra was \(270\text{mg.100ml}^{-1}\) \((67.5\text{mM.1}^{-1})\) during vitellogenesis which is within the range of values reported in Thamnophis saurits \((90 \text{mM.1}^{-1})\) and Sceloporus cyanogenys \((112 \text{mM.1}^{-1})\) near ovulation. These values are far in excess of values reported for the cobra, Naja naja \((34.08 \text{mM.1}^{-1})\) and Dipsosaurus dorsalis \((3.73 \text{mM.1}^{-1})\). The turtle, Chrysemys picta, shows less dramatic increases during the preovulatory stages \((4.07 \text{mM.1}^{-1})\) (Callard et al 1978). Clark (1967) recorded similar elevations for the same species during the breeding period \((3.83 \text{mM.1}^{-1})\), however, supranormal levels were recorded following oestradiol therapy. These results suggest
that species differences with respect to plasma calcium levels exist in vitellogenic reptiles. In vitellogenic females of other oviparous vertebrates, elevations in plasma calcium have been demonstrated: teleosts (Woodhead 1969), the amphibian, *Xenopus laevis* (13 fold increase) (Pollet & Redshaw 1968) and birds (Riddle et al 1944; Simkiss 1967). However, no significant changes in calcium concentration could be detected in elasmobranchs. (Craik 1978).

Analysis of calcium in vertebrate plasma has shown that it exists in two fractions described as the diffusible and the non-diffusible forms. Furthermore, diffusible calcium consists mainly of ionic calcium together with small amounts of un-ionized calcium (mostly in combination with inorganic anions). Non-diffusible calcium on the other hand is protein bound, mainly to albumins. It is a known fact that the ionic calcium is the physiologically active component. The relationship between the diffusible and non-diffusible calcium has been considered to be an equilibrium, based upon the dissociation of a weak electrolyte of calcium proteinate (Simkiss 1967). Therefore, providing that the total protein concentration and the ability of proteins to bind calcium does not change, the ratio of ionized to un-ionized calcium should remain approximately constant (Simkiss 1967). Recording variations in total plasma calcium seems meaningful because they are directly proportional to the ionic calcium concentration in the blood. However, it should be stressed that estimates of the total blood calcium concentration with no corresponding estimate of the degree of ionization may not be a true reflection of the calcium status (Landerson et al 1973 cf. Luck & Scanes 1978).

Vitellogenin is reported to be a complex calcium binding lipophosphoprotein, present in the plasma of vitellogenic females of most oviparous animals. Therefore, the appearance of vitellogenin in the blood of oviparous animals
could be expected to increase the non-diffusible calcium component. However, Urist et al (1958) established that the yolk protein is deposited in the ovarian follicles with the full compliment of calcium with which it was associated in the blood. This would then imply that the production of yolk puts no stress upon ionized calcium. Presumably, changes in the ionized calcium levels represent changes in both the demand for shell synthesis and for supply from the gut and bone (Luck & Scanes 1978).

Follet & Redshaw (1974) reported that the vitellogenin yolk precursor found in the plasma of amphibians shows relatively low affinity towards calcium and therefore no extreme calcium stress could be recorded. However, considering the calcium richness of the yolk and the large number of cleidoic eggs being produced simultaneously in reptiles, a large drain upon calcium reserves could be expected. Dessauer et al (1956) recorded surprisingly high rate of calcium metabolism in the ribbon snake (4mg kg$^{-1}$ bodymass$^{-1}$). Moreover, calcium metabolism studies suggest that the turtle, Sternothaenus odoratus, passes through a period of calcium stress during the reproductive season. However, normal bone resorption appeared to be sufficient to meet the needs of reproduction (Simkiss 1967). Similar conclusions have been reached by Suzuki (1963) following a histological investigation on bone resorption in the turtle, Pseudemys scripta elegans. There was no evidence for any medullary bone formation and resorption primarily came from a thinning of the trabeculae.

It is however among the squamata that remarkable adaptations in calcium metabolism are found. The endolymphatic sacs of anoline and geckonid lizards are reported to contain a suspension of calcium carbonate, probably a readily releasable calcium store during stress situations. (Jenkins & Simkiss 1968; Dessauer 1970). Little is known about the mechanism
whereby the calcium is laid down or withdrawn from these sacs but it has been suggested that the female hormone, oestradiol, activates the process. (Simkiss 1967). To this extent the sacs may be utilized in an analogous way to medullary bone in birds.

In the present study, X-ray opacity studies performed with female *Agama atra* did not show any calcium carbonate in the endolymphatic sacs, suggesting a possible calcium metabolism similar to birds or chelonians. Unfortunately histological investigations of the formation of medullary bone in the female *Agama atra* did not throw any light on this question because of artifacts occurring during histological preparations and sectioning. Further study is needed to investigate the calcium metabolism of *Agama atra*.

4.3.5. Variation in the hepatosomatic index

The importance of the liver in the reproduction of the oviparous animals has been well documented (Dessauer 1955; Telford 1970; Goldberg 1972; Gerstle & Callard 1972; Callard et al 1972c; Lance 1975; Lin 1979).

Vitellogenesis accompanied by liver hypertrophy suggests that the liver is the site of several vitellogenic precursors, in birds (Heald & McLachlan 1965; Schjeide et al 1963) in amphibians (Follet & Redshaw 1968) in the turtle (Chaikoff & Enterman 1946; Callard et al 1978) in snakes (Lance 1975) in lizards (Yaron & Widzer 1978) and in the general review by Dessauer (1974).

Considerable variation in the liver index occurred throughout the year (figure 62). No significant change was evident during the vitellogenic stages. The absolute liver mass recorded during the first vitellogenic stage however indicated signs of liver hypertrophy (figure 62). During the second vitellogenic cycle a similar trend was observed. Noteworthy, was that the highest individual liver mass was recorded during May (1.0569 gram),
Figure 62. Variation in the hepatosomatic index and absolute liver mass during the reproductive cycle of the female Agama atra. Symbols as in figure 54.
July (1.1276 gram) and Pre-ovulatory I (1.1227g) whereas the lowest was recorded in August (0.2937 gram).

Lance (1975) reported a similar liver hypertrophy during the previtellogenic and vitellogenic stages, followed by a reduction in liver mass in the cobra, Naja naja during the winter months. Similar trends were reported for the lizards, Sceloporus jarrovi (Goldberg 1972), Tachydromus tachydrromaoides (Telford 1970), Chamaeleo hohneli and Chamaeleo jaksoni (Lin 1979). Telford (1970) recorded a preovulatory reduction in liver mass which continued during the development of subsequent clutches. He suggested that adult females rely primarily upon their fatbodies for production of the first clutch, whereas the liver reserves are utilized for later clutches. The present results were found to be inconsistent with these observations since a further liver hypertrophy possibly occurs during the second vitellogenic stage. However, further investigation is needed to elaborate this suggestion.

In birds direct evidence based on functional hepatectomy, isotopic tracer studies and other methods indicated that the liver is the site of synthesis of most if not all the major yolk precursors. (Vanstone et al 1957; Greengard et al 1965). In reptiles the liver has also been suggested to be the site of yolk precursor synthesis. (Hahn 1967; Dessauer & Fox 1956; Callard et al 1972c; Gerstle & Callard 1972; Yaron & Widzer 1978).

Furthermore, radio-isotopic studies performed in the lizard, Uta stansburiana, indicated that oestrogens increased liver R.N.A. synthesis (Hahn & Tinkle 1965).

It is generally assumed that ovarian hormones (mainly oestrogens) induce hepatic synthesis of vitellogenin (yolk precursor) and that the concomitant hepatic growth requires the presence of the intact pituitary gland. (Mainly growth hormone) (Callard et al 1972 a and b). The hepatic hypertrophy following oestradiol therapy in the male Agama atra may corroborate this suggestion (Figure 66). The quantitative increase in liver size may, however,
not necessarily solely reflect elevations in nucleic acids, proteins or increased glycogen levels, but also alterations in lipid content and metabolism (Callard et al 1972c). Moreover, Callard et al (1972c) indicated that an elevation in the number of cells in the liver occurred in Dipsosaurus dorsalis although the total liver size did not change. The liver mass does therefore not necessarily correlate with synthetic activity in the liver, which may be a possible reason for poor seasonal trends in the liver index found in the female Agama atra.

4.3.6. Variation in oviducal index

The seasonal variations in the oviducal index (g. 100g bodymass$^{-1}$) is presented in figure 63. Outside the breeding season the oviducts regressed in size and became thin collapsed tubules. Hypertrophy of the oviducts coincided with ovarian recrudescence and the highest index values were recorded during the postovulatory stages ($P<0.001$; May vs Postovulatory I). It was noteworthy that the oviducal index did not change significantly between two vitellogenic cycles within the breeding season. ($P<0.5$ Postovulatory I vs Postovulatory II).

Lizards in general have paired oviducts, each differentiated along its length into clearly distinguishable sections (Fox 1977). Tubular glands (albumin and shell secreting glands) were reported to show seasonal changes in size (Wiedersheim cf. Fox 1977).

The season oviducal hypertrophy observed in the female Agama atra was in accordance with most of the reports on lizards and snakes (Fox & Dessauer 1962; Callard et al 1972b; Yaron 1972; Burrage 1973; Lance & Callard 1978; Botte 1973 a & b; Nilson 1981). In contrast to Agama atra it has been reported that the oviducts of Acanthodactylis regressed between successive ovarian cycles during the breeding season (Bons 1972 cf. Fox 1977). Furthermore, the oviducts of the female lizard, Uma scoparia, remained in a hypertrophied condition throughout life (Mayhew 1966).
Figure 63.

Variation in the oviducal index during the reproductive cycle of the female *Agama atra*. Symbols as in figure 54.
Limited information exists on the biochemical changes that take place in the oviduct of oviparous reptiles during the reproductive cycle. Botte (1973a) reported that the oviduct of *Lacerta sicula* showed considerable hypertrophy in all regions especially in the tubal and uterine glands. Furthermore, the protein content, total RNA and activity of acid and alkaline phosphatases increased simultaneously with hypertrophy. The activity of oviducal glycogen and β-glucuronidase increased concomitantly with increased ovarian steroidogenesis in several snakes. (Callard & Hirsch 1967, 1970a).

The influence of the ovary on the oviduct has been demonstrated in several reptilian species. Oviducal regression followed ovariectomy in several lizards and snakes. Furthermore, oestrogen replacement therapy restored oviducal condition, whereas prolonged treatment resulted in oviducal hypertrophy similar to seasonal breeding females. Similar effects were obtained after steroid treatment in female lizards of intact non-breeding females (Prasad & Sanayal 1969; Callard 1970a; Yaron 1972; Veith 1974).

Callard & Klots (1973a) indicated that oestrogen treatment resulted in oviducal growth in several vertebrate species and therefore suggested that the oviducal index may be used as an indirect indication of oestrogenic activity. Confirmation of his suggestions came from seasonal studies of plasma steroids in the turtle, *Chrysemys picta*, which indicated that seasonal steroid cycles coincided with the oviducal cycles (Callard et al 1978). Using the correlation between oviducal growth and oestrogen levels (Callard & Klots 1973a) it then seems clear that oestrogen levels coincided with the onset of vitellogenesis in the female *Agama atra* (Compare figures 9 and 63).

The role of progesterone during oviducal hypertrophy and maintenance is not clear. However, it has been suggested that both oestrogen and progesterone are essential for oviducal maturation (Yaron 1972; Veith 1974). On the other hand an interaction of 17-β oestradiol and testosterone in stimulating the oviduct of the lizard, *Lacerta sicula*, has been reported (Botte et al 1974).
It is therefore clear that much more information on the characteristics of the steroids and their possible interactions in the reptilian oviduct is needed.

4.3.7. Variation in plasma progesterone

The basic mammalian pattern of steroid hormone biosynthesis is present in the ovaries of all vertebrates, with the possible exception of some teleost species (Lance & Callard 1978). The role of progesterone in non-mammalian vertebrates is not clear, although there are data to suggest that in some species this steroid is secreted by the preovulatory follicle (Callard et al. 1978) just before ovulation whereas several reports suggest a corpus luteum origin after ovulation (Callard et al. 1972a; Callard & Doolittle 1973b; Veith 1974; Highfill & Mead 1975a). The object of the present investigation was to establish the basic progesterone patterns in the plasma, correlated with the standard morphological and gravimetric changes associated with the reproductive cycle.

Variation in plasma progesterone concentration during the reproductive cycle of *Agama atra* is presented in figure 65. It may be observed that the plasma progesterone levels did not show considerable variation outside the breeding season, ranging between 350 and 660 pg.0.2ml\(^{-1}\) and likewise during vitellogenesis relatively low progesterone levels were recorded. (660 - 900 pg.0.2 ml\(^{-1}\)). However, following ovulation, significantly increased plasma progesterone levels were recorded in the presence of functional corpora lutea. (P<0.001, Preovulatory vs Postovulatory I and II). A significant decrease in plasma progesterone was associated with the second pre-ovulatory stage, although remnants of corpora lutea were present. The second post-ovulatory was characterized by significantly increased progesterone levels in the presence of functional corpora lutea. An evident decline in plasma progesterone concentration followed the second oviposition cycle and persisted throughout the non-breeding season.
Standard concentration curve used to determine total plasma progesterone concentration. $r =$ correlation coefficient; $s =$ slope of the regression line.
Figure 65.
Variation in the total plasma progesterone concentration during the reproductive cycle of the female Agama atra. Symbols as in figure 54.
The present investigation in the oviparous Agama atra indicates that the plasma levels of progesterone are markedly elevated in females containing oviducal eggs and functional corpora lutea (good blood supply). The observed fluctuations in plasma progesterone levels of the female Agama atra seems therefore to indicate a correlation with the appearance of the corpora lutea and their involution following oviposition. A similar increase in plasma progesterone levels, following ovulation, has been reported in the agamid lizard, Uromastix hardwicki (Arslan et al 1978). Likewise progesterone levels in other squamates rise after ovulation and remain high as long as functional corpora lutea persist (Table 4). Unlike in most squamates, a major progesterone surge prior to ovulation was recorded in oviparous turtles, Chrysemus picta (Callard et al 1978) and Chelonia mydas (Lance & Callard 1978). Progesterone levels decline after ovulation, reaching basal levels only after oviposition in these turtles. Lance & Callard (1978) pointed out that vitellogenic cycles of squamates and Cheloniids differ considerably although the hormones controlling the process are probably similar. The association of high progesterone levels with the maturation of ovarian follicles in the oviparous turtles is in contrast to most squamates and this condition is regarded as primitive in the evolution of oviparous reproductive mechanisms by Callard et al (1978). Licht et al (1979a) recorded a similar ovulatory progesterone surge of about 24 hour duration, whereafter the plasma progesterone rapidly returned to baseline levels, indicating that the corpora lutea were relatively inactive after ovulation. Lewis et al (1979) did not record the preovulatory progesterone surge in Chelonia mydas, probably because the transient nature of this progesterone surge (Callard et al 1978).

The plasma progesterone concentrations recorded for Agama atra are somewhat higher than those observed in viviparous lizards, Sceloporus jarrovi (Guilette et al 1981), Sceloporus cyanogenus (Callard et al 1972b), Chamaeleo pumilus (Veith 1974) and the snakes, Natrix taxispilola (Lance & Callard 1978) and Thamnophis elegans (Highfill & Mead 1975a and Mead et al 1981) (Table 4). The plasma progesterone levels outside the breeding season measured in the
present study were much higher than reported for the oviparous lizard, *Uromastix hardwicki* (Arslan et al 1978) and the oviparous snake, *Naja naja* (Bona-Gallo et al 1980). The pre-ovulatory levels in *Agama atra* correspond to the levels of the above mentioned lizard and snake whereas the post-ovulatory levels were lower than recorded in *Uromastix* and *Naja* (Table 4). Highfill & Mead (1978a) attributed difference in plasma progesterone levels to species differences in reptiles.

The persistence of elevated progesterone levels after ovulation in *Agama atra* suggests, unlike those of some turtles, that the corpora lutea remains functional until the time of oviposition. These observations are consistent with most oviparous squamata, although the viviparous mode is characterized by prolonged progesterone elevation or corpora lutea activity (Table 4). Moreover, Arslan et al (1978) indicated that progesterone content of the luteal tissue in *Uromastix hardwicki* was significantly higher than that of the follicular tissue. Similarly, several studies indicated that the corpora lutea in ovo-viviparous and viviparous snakes and lizards are endocrine glands which secrete progesterone (Browning 1973). Chan & Callard (1965) demonstrated the conversion of pregnenolone to progesterone in the corpus luteum of the snake, *Natrix*. Several workers demonstrated the presence of the enzyme $\Delta^5$-3-beta-hydroxysteroid dehydrogenase (3β - HSD) which is directly involved in the synthesis of progesterone in the corpora lutea of viviparous reptiles. (Callard 1966; Morat 1969; Highfill & Mead 1975b; Callard et al 1972a; Yaron 1972). Moreover, progestins have been identified in the corpora lutea of ovo-viviparous snakes, *Crotalus* and *Bothrups* (Porta 1942) and the activity of 11-deoxy-cortisosterone and 21-hydroxylase in the luteal tissue of the snake, *Storeria dekayi*, and the lizard *Xantusia vigilis* (Colombo et al 1974, 1976). Klicka & Mahmoud (1972) reported that the corpora lutea of the turtle, *Chelydra serpentina*, converted pregnenolone $^{14}$C to progesterone $^{14}$C. Although these studies suggest the endocrine nature of the corpora lutea in viviparous reptiles, they failed to show
### TABLE 4: PLASMA PROGESTERONE LEVELS (ng. ml⁻¹) DURING THE REPRODUCTIVE CYCLE OF SOME REPTILES (MEAN ± SD)

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>PREVITELLOGENIC STAGE</th>
<th>PREOVULATORY STAGE</th>
<th>POSTOVULATORY STAGE</th>
<th>LATE STAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysemys picta¹</td>
<td>0.97 ± 0.064</td>
<td>5.004 ± 1.019</td>
<td>0.457 ± 0.012</td>
<td>-</td>
</tr>
<tr>
<td>Chelonia mydas²</td>
<td>0.173 ± 0.084</td>
<td>1.758 ± 0.130</td>
<td>0.678 ± 0.88</td>
<td>-</td>
</tr>
<tr>
<td>Chelonia mydas²</td>
<td>1.82 ± 0.29</td>
<td>3.00 ± 0.38</td>
<td>-</td>
<td>Oviparous</td>
</tr>
<tr>
<td>Chelonia mydas³</td>
<td>1.90 ± 0.50</td>
<td>2.36 ± 0.29</td>
<td>-</td>
<td>Oviparous</td>
</tr>
<tr>
<td>Chelonia serpentina⁴</td>
<td>0.25 ± 0.07</td>
<td>0.31 ± 0.11</td>
<td>1.44 ± 0.32</td>
<td></td>
</tr>
<tr>
<td>Sceloporus cyanogenys⁵</td>
<td>0.700 ± 0.15</td>
<td>0.900 ± 0.38</td>
<td>3.302 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>Scleoporus jarrovi⁶</td>
<td>± 0.7</td>
<td>± 1.00</td>
<td>3.78 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>Uromastix hardwicki⁷</td>
<td>1.660 ± 0.300</td>
<td>13.410 ± 1.430</td>
<td>-</td>
<td>Oviparous</td>
</tr>
<tr>
<td>Chamaeleo pulilus⁸</td>
<td>0.864</td>
<td>0.9456 ± 0.713</td>
<td>4.946 ± 3.902</td>
<td>2.296 ± 0.338</td>
</tr>
<tr>
<td>Agama atra⁹</td>
<td>2.139 ± 0.500</td>
<td>2.976 ± 0.999</td>
<td>7.450 ± 1.724</td>
<td></td>
</tr>
<tr>
<td>Naja naja¹⁰</td>
<td>± 1.4</td>
<td>± 3.00</td>
<td>10.5 ± 11.25</td>
<td></td>
</tr>
<tr>
<td>Natrix taxispilota⁻²</td>
<td>0.442 ± 0.040</td>
<td>0.012 ± 0.084</td>
<td>1.925 ± 0.241</td>
<td></td>
</tr>
<tr>
<td>Natrix sipedon¹¹</td>
<td>1.270 ± 0.19</td>
<td>3.930 ± 0.83</td>
<td>4.950 ± 1.41</td>
<td>6.940 ± 0.78</td>
</tr>
<tr>
<td>Thamnophis elegans¹²</td>
<td></td>
<td>1.700 ± 0.30</td>
<td>6.200 ± 1.00</td>
<td></td>
</tr>
<tr>
<td>Thamnoplis elegans¹³</td>
<td></td>
<td>3.3 ± 2.3</td>
<td>3.9 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

that the corpora lutea are the major source of plasma progesterone. The
demonstration of Δ⁵-3β-HSD in the corpus luteum does not prove that the
major function of the organ is progesterone synthesis. Additional
investigations are necessary to determine whether all the enzymes required
for steroidogenesis are present. (Klicka & Mahmoud 1972). Moreover,
Klicka & Mahmoud (1972) demonstrated the conversion of cholesterol to
progesterone. High progesterone content of the luteal tissue was reported
in the viviparous snakes, Thomophis elegans (Highfill & Mead 1975a) and
viviparous lizard, Chamaeleo pumilus pumilus (Veith 1974) and the oviparous
however, reported an inverse correlation between corpus luteum diameter and
plasma progesterone in the viviparous lizard, Sceloporus jarrovi, suggesting
that the corpora lutea were not the major source of progesterone during
gestation.

Another possible source of elevated plasma progesterone may be the adrenal
gland, as shown for the cobra, Naja naja (Huang et al 1969). Highfill & Mead
(1975b) presented results indicating that the adrenals of snakes synthesize
progesterone as suggested by Callard & Leathen (1964) and being secreted
into the bloodstream (Chan & Callard 1973). However, Highfill & Mead (1975a)
concluded that the adrenals were the major source of low levels of progesterone
in the non-pregnant snakes. Moreover, Callard et al (1978) reported that
injections of ACTH significantly increased plasma progesterone levels in
castrates, whereas FSH did not, thereby suggesting the progesterone levels
during the non-breeding season may originate from the adrenals.

Although no positive correlation between numbers of corpora atretica and
plasma progesterone could be detected in Agama atra, Guillette et al (1981)
suggested an endocrine function of these structures in the viviparous
lizard, Sceloporus jarrovi. Moreover, Nandi (1967) indicated that reptilian
atretic follicles reacted positively for the steroidogenic enzyme \( \Delta^5 \)-3-\( \beta \)-HSD. Several workers reported corroborating evidence for the endocrine role of corpora atretica in reptiles (Guraya & Varma 1976; Guraya 1976; Byskov 1978; Saidapur 1978; Gouder et al 1979). Moreover, Guilette et al (1981) observed a positive correlation between numbers of atretic follicles and monthly plasma progesterone with inhibited ovarian responses in the lizard, *Anolis carolinensis*. Bona-Gallo et al (1980) however, suggested that corpora atretica in the cobra, *Naja naja* made little contribution to circulating progesterone levels. It is apparent from the above discussion that whether atretic follicles have any steroidogenic potentiality or not has not been resolved. Some investigators feel that the presence of \( \Delta^5 \)-3-\( \beta \)-HSD and other steroidogenic enzymes in early atretic follicles may be either remains or contamination of the pre-existing activity of the granulosa cells. Further study is evidently needed in this respect (Saidapur 1978).

The functional significance of the corpora lutea during the reproductive cycle of *Agama atra* remains enigmatic, especially because the onset of vitellogenesis was evident in the presence of functional corpora lutea. Although it seems evident that the corpus luteum secretes progesterone, it is yet unknown whether progesterone functions as a hormone as in mammals. Several investigators have postulated a variety of functions for progesterone in regulating reptilian reproductive physiology. These included presenting or retarding the rate of ovarian follicular development (Callard et al 1972a; Browning 1973; Veith 1974; Mahmoud & Klicka 1972; Callard & Klots 1973a). Callard et al (1972a) postulated that progesterone inhibits the biosynthesis of oestrogen and thereby reduces vitellogenesis or otherwise blocks reactions essential to vitellogenesis. The present investigation, however, cannot corroborate these assumptions, because of the occurrence of vitellogenesis in the presence of relatively high plasma progesterone levels in *Agama atra*. Yaron & Widzer (1978) suggested that progesterone inhibits the incorporation of vitellogenin into the oocytes either directly or by antagonizing the stimulatory effect of gonadotropins.
This suggestion still does not explain the ovarian growth observed in the presence of high progesterone levels as observed in the female *Agama atra*.

Roth et al (1973) reported that deluteinization in the oviparous lizard, *Sceloporus undulatus*, resulted in a significant reduction in oviposition time. Similarly, premature oviposition was induced in the oviparous lizard, *Chemidophorus uniparens* (Cuellar 1979). Ovariectomy performed in the turtle, *Chrysemys picta*, also resulted in premature oviposition (Klicka & Mahmoud 1977). Several ovariectomy and hypophysectomy experiments performed on viviparous lizards and snakes, resulted in abortion or resorption of embryos. (Clausen 1940; Rahn 1938; Yaron 1972; Burrage 1973; Veith 1974). However, a number of studies indicated that neither ovariectomy nor luteectomy did interfere with the maintenance of pregnancy in ovoviviparous and viviparous reptiles (Bragdon 1951; Badir 1968; Callard et al 1972a; Highfill & Mead 1975b). Highfill & Mead (1975b) pointed out that most of the previous studies have failed to deal directly with corpora lutea, or did not know the exact time of corpora lutea formation which may lead to conflicting data. The present study indicates that it remains inconclusive whether progesterone is involved in regulating the duration of pregnancy and more study is needed to elucidate the influence of this hormone on vitellogenesis.

It is suggested that progesterone may influence glandular development or vascularity of the oviduct in reptiles. Several workers suggested that the seasonal changes in the oviduct may be endocrine induced, especially by luteal hormones. (Callard 1965). Ovariectomy and subsequent steroid replacement were found to indicate that progesterone may be necessary for oviducal maintenance during gestation. (Yaron 1972). Likewise, Veith (1974) has reported that oestrogens stimulate the mucosa of the oviduct of the lizard, *Chamaeleo pumilus*, and that progesterone acts synergistically with oestrogen.
in this regard. Botte (1974) essentially indicated a similar synergism in *Lacerta sicula*. In the present study it was evident that oviducal hypertrophy was present throughout the breeding season although plasma progesterone only reached high levels during the post-ovulatory stages. It seems therefore that oviducal hypertrophy is independent of progesterone. Mead et al. (1981) studied the effects of both progesterone and oestradiol on the histology of the oviduct in the snake, *Thamnophis elegans*, and clearly indicated that oestrogen is the most important ovarian hormone in regulating oviducal growth. Their results indicated that no conclusive role for progesterone in altering oviducal histology could be reached nor did they find a progesterone-oestradiol synergism. However, additional hormones, other than progesterone were suggested to be required for full restoration of the snake oviduct to the preovulatory condition.

The role of steroids in the control of oviducal contraction has only been investigated by La Pointe (1969) using the lizard, *Klauberina riversiana*. However, in this study, neither oestradiol nor progesterone were shown to modify oviducal contractions. Callard & Hirsch (1976), however, indicated that progesterone significantly reduced the duration of both contractile and resting periods of the oviduct in the turtle, *Chrysemys picta*. Several workers proposed that steroids may influence the response of neurohypophysial hormones on the oviduct (Heller 1972; Callard & Hirsch 1976; Guillette 1979). It seems evident that more elaborate investigation is needed to determine whether progesterone plays any functionally significant role in controlling oviducal contractions in reptiles.

Moreover it remains to be determined if progesterone is functionally associated with the reproductive control system of the oviparous lizard.

4.4. **Conclusions**

1. Lipids were mainly stored in paired abdominal-situated corpora adiposa. A typical negative correlation between fatbody index and ovarian growth
was evident during the breeding season. The production of the first clutch is suggested to be dependent on the presence of large fatbodies observed during the winter months.

2. Cholesterol levels remained relatively low during vitellogenesis which may indicate increased ovarian uptake. Further research is needed to elucidate the role of cholesterol in the reproduction of non-mammalian vertebrates.

3. The plasma proteins of the female *Agama atra* resolved into five major fractions upon electrophoresis. Fraction II (β-globulin mobility) increased significantly during vitellogenesis which may be the result of increased production of the yolk precursor, vitellogenin. The total protein concentration showed considerable individual variation, but generally an inverse correlation between TPP and the gonadosomatic index was evident.

4. The highest mean calcium concentration was recorded during vitellogenesis. It may indicate that marked calcium turnover occurred during this period associated with the production of the yolk precursor, vitellogenin.

5. Poor seasonal trends in the hepatosomatic index may indicate that the liver mass does not necessarily correlate with synthetic activity of the liver.

6. Hypertrophy of the oviduct coincided with ovarian recrudescence which may indicate the trophic activity of ovarian hormones on the oviduct.

7. Progesterone levels were elevated during the post-ovulatory stages until oviposition occurred. The strong correlation with the presence of large functional corpora lutea may indicate the functional significance
of these organs in synthesizing progesterone.
CHAPTER 5

HORMONAL INDUCED VITELLOGENESIS IN THE MALE AGAMA ATRA

5.1. Introduction

The induction of vitellogenesis in both female and male non-mammalian vertebrates using steroid therapy has been widely reported. The rate and magnitude of the vitellogenic response was found to be dependent upon the amount of hormone injected (Dessauer 1970). The major changes in plasma components during vitellogenesis are now known to be mainly the result of increase levels of vitellogenin, a calcium-binding lipophosphoprotein. There is much evidence to show that vitellogenin is produced in the liver (Callard et al 1972c). It is furthermore evident that vitellogenin synthesis is under the influence of oestrogenic hormones. (Wallace & Jared 1968). The present investigation was undertaken in order to determine the importance of oestrogen during the onset of vitellogenesis and whether a synergism between oestrogen and progesterone exists.

5.2. Procedure

5.2.1. Collecting and housing

Mature male lizards (SVL = 8.46 ± 0.99 cm) were collected during January 1980 at De Kelders (latitude: 34° 33'S and longitude: 19°21'E) as previously described. (Par. 2.2.2.). After toeclipping, the lizards were grouped randomly into four groups of four lizards. Each group was housed in a separate polyethylene cage (40 x 40 x 30 cm), kept inside a controlled climatic room for the duration of the experiment. They were subjected to a constant temperature minimum of 17.8 ± 0.23°C during a 10 hour light period, fed Tenebrio molitor larvae and received water ad. libitum.

5.2.2. Hormonal treatment and autopsy

After a seven day acclimatization period the lizards received the following
intraperitoneal injections every second day for a period of 22 days:

**Group 1**: Oestradiol cypionate, dissolved in cottonseed oil at a dose of 50 µg oestradiol \( \cdot \) 100 gram body mass \(^{-1} \).

**Group 2**: Progesterone (\(\Delta^4\)-Pregnen-3-20-dione) dissolved in cottonseed oil at a dose of 5 µg progesterone \( \cdot \) 100 gram body mass \(^{-1} \).

**Group 3**: Oestradiol cypionate (50 µg, 100 gram bodymass \(^{-1} \)) plus progesterone (50 µg \( \cdot \) 100g \(^{-1} \)).

**Group 4**: Pure cottonseed oil (50 ml.100g bodymass \(^{-1} \)).

One day after the last injection the lizards were killed and bloodsamples collected as previously described (par. 2.2.3.) centrifuged at 2 000 r.p.m. and the plasma frozen for subsequent analysis. The testes, liver and fat= bodies were cleaned of adherent tissue and weighed to the nearest 0.0001 gram. All biochemical analyses were performed as previously described (par. 2.2.3.).

5.3. Results and discussion
The results in which hormones were injected for 22 days are presented in table 5 and figure 66.

All plasma constituents measured were significantly higher than in controls, after receiving oestradiol cypionate injections every second day for 22 days. (\(P< 0.001 \)). Males treated with progesterone did not show any significant changes when compared to the control group (\(P>0.05 \)). Furthermore, all plasma constituents measured in the males after receiving oestradiol plus progesterone were significantly higher than both control and progesterone treated groups (\(P< 0.001 \)). The hepatosomatic index of males treated with oestradiol was significantly higher than the other groups (\(P< 0.001 \), Group I vs Control). The fatbody index, however, did not show any significant changes in any of the groups.
FIGURE 66.

The presence of a new plasma protein with relatively low mobility after oestrogen treatment to male *Agama atra.*
### TABLE 5. THE EFFECT OF OESTROGEN INJECTION ON THE LIVER INDEX, FATBODY INDEX, PLASMA CALCIUM, CHOLESTEROL, TOTAL PROTEINS AND TOTAL LIPIDS IN THE MALE AGAMA ATRA (MEAN + SD)*

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>n</th>
<th>Testis Index</th>
<th>Liver Index</th>
<th>Fatbody Index</th>
<th>Plasma Calcium</th>
<th>Plasma Cholesterol</th>
<th>Total Protein</th>
<th>Total Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(g.100g⁻¹)</td>
<td>(g.100g⁻¹)</td>
<td>(g.100g⁻¹)</td>
<td>(mg.100ml⁻¹)</td>
<td>(mg.100ml⁻¹)</td>
<td>(mg.100ml⁻¹)</td>
<td>(mg.100ml⁻¹)</td>
</tr>
<tr>
<td>CONTROL</td>
<td>4</td>
<td>0.62 + 0.37a</td>
<td>2.54 + 0.35</td>
<td>0.75 + 0.05</td>
<td>4.05 + 1.60e</td>
<td>295 + 1.60b</td>
<td>8.13 + 1.60</td>
<td>19.5 + 7.5</td>
</tr>
<tr>
<td>OESTROGEN</td>
<td>4</td>
<td>0.52 + 0.19</td>
<td>4.62 + 0.39</td>
<td>0.83 + 0.10</td>
<td>25.7 + 6.40f</td>
<td>586 + 50.0f</td>
<td>23.7 + 4.60</td>
<td>50.0 + 6.0</td>
</tr>
<tr>
<td>PROGESTERONE</td>
<td>4</td>
<td>0.47 + 0.03a</td>
<td>2.45 + 0.34a</td>
<td>0.57 + 0.06d</td>
<td>8.60 + 0.20e</td>
<td>221 + 31.7b</td>
<td>7.20 + 1.08a</td>
<td>11.3 + 3.2a</td>
</tr>
<tr>
<td>PROGESTERONE</td>
<td>4</td>
<td>0.38 + 0.11a</td>
<td>3.51 + 0.36d</td>
<td>0.16 + 0.04f</td>
<td>15.03 + 1.06f</td>
<td>428 + 60.0e</td>
<td>15.6 + 1.16f</td>
<td>50.67 + 8.0f</td>
</tr>
</tbody>
</table>

* Student's t probability (p) values after compared with control
  
a. P > 0.01 not significant different
b. P < 0.05 not significant different
c. P < 0.02 different
d. P < 0.01 Significant different
e. P < 0.002 Significant different
f. P < 0.001 Highly significant different.
Treatment of *Agama atra* males with oestradiol resulted in hepatic hypertrophy. A similar response has been reported in other reptiles (Callard et al. 1972c; Gerstle & Callard 1972; Lance 1975; Yaron & Widzler 1978). Concomitant with the hepatic hypertrophy was the appearance of a new protein component in the plasma (figure 66). However, the relative concentration and electrophoretic mobility of this component could not be ascertained because of insufficient separation during cellulose acetate electrophoresis. Dilution of sample caused precipitation and extraction of lipoproteins was insufficient, however, figure 66 indicates clearly the appearance of a new plasma protein component. The electrophoretic mobility of this new protein fraction seems comparable to the mobility of the new protein fraction observed in the female *Agama atra* and other previously reported mobilities for vitellogenin in other reptilians (Hahn 1967; Suzuki & Prosser 1968; Gerstle & Callard 1972; Veith 1974; Yaron & Widzler 1978).

The addition of progesterone to oestradiol treatment did not prevent the appearance of plasma vitellogenin, while the administration of progesterone alone did not lead to the appearance of plasma vitellogenin. These results correspond to observations reported in the lizards, *Xantusia vigilis* (Yaron & Widzler 1978) and *Chamaeleo pumilus pumilis* (Veith 1974). The highly significant elevations in total plasma protein, calcium, lipid and cholesterol concentrations following oestradiol and oestradiol plus progesterone therapy, corroborate the suggestion of increased vitellogenin production in *Agama atra*. Similar elevations in plasma calcium were reported in the cobra, *Naja naja* (Lance 1975). Furthermore, supranormal calcium levels following oestradiol treatment were also reported in lizards, *Dipsosaurus dorsalis* (Gerstle & Callard 1972). *Sceloporus cyanogenys* (Suzuki & Prosser 1968) and turtles (Clark 1967). Simkiss (1962) suggested that calcium was necessary for the proteins to remain soluble in the serum. This would explain why hypercalcaemia took place even when no outside source of calcium was available. Dessauer
et al (1959) suggested that the calcium is released from the bone, but, no apparent bone resorption could be detected in Sceloporus cyanogenys (Suzuki & Prosser 1968). Evidently, further study is necessary to elucidate this problem.

Elevations in plasma cholesterol during oestradiol treatment is in contrast to findings after similar treatment in the cobra, Naja naja (Lance 1975). Moreover, Lance (1975) suggested that the hormone or hormones controlling the metabolism of plasma calcium and plasma cholesterol may be different. Dessauer (1955) reported a marked turnover and exchange of lipids between the liver and the fatbody. However, no significant increase or decrease in fatbody index may suggest additional control mechanisms. Increased plasma cholesterol levels may be ascribed to increased cholesterol synthesis in the liver and subsequent release into the bloodstream.

The role of progesterone as an "antigonadal" hormone was suggested in a review on the endocrine control of the reptilian gonad (Callard et al 1972a). They suggest an inhibitory action on the synthesis of ovarian oestrogens, consequently reducing vitellogenesis or associated critical reactions either in the liver or fatbody or both. The above results clearly show, however, that progesterone did not reduce the effect of oestrogens on the liver when administered alone, and no significant effect on the liver could be detected. Yaron & Widzler (1978) suggest that the antigonadal effect on progesterone is probably exerted at follicular level, consequently inhibiting vitellogenin uptake into the oocyte and thereby resulting in an accumulation of vitellogenin in the plasma and no ovarian growth. However, in the present seasonal investigation ovarian growth was observed in the presence of high progesterone levels in the female Agama atra. This observation therefore questions the antigonadal effect of progesterone in oviparous lizards and indicate that more elaborate investigation is needed in this regard.
5.4. Conclusions

1. Oestradiol and oestradiol plus progesterone treated males showed a highly significant increase in all the measured plasma constituents associated with vitellogenesis.

2. Evidently a new plasma protein appeared, with relatively low mobility suggesting a high molecular mass.

3. Neither a synergistic effect between oestradiol and progesterone nor an inhibitory effect of progesterone was evident.
CHAPTER 6

GONADOTROPIN STIMULATION OF OVARIAN AND OVIDUCAL GROWTH AND OVULATION IN THE

FEMALE AGAMA ATRA.

6.1. Introduction
The stimulatory effect of injections of mammalian gonadotropins on the reproductive system of reptiles has been reported for a number of reptilian species. However, the majority of these reports have been concerned with the response of the male system to gonadotropins. Furthermore, very few of these reports cited dealt with squamate gonadotropins.

Several lines of evidence indicate that the physiological roles of the reptilian pituitary gonadotropins, especially in squamates, may differ considerably from those observed in mammals and other vertebrates (Licht & Paphoff 1974b). The existence of two separate gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH), with separate physiological actions are known for eutherian mammals. Early workers suggested a single gonadotropin complex for reptiles, but recent fractionation studies have revealed the presence of distinct FSH and LH molecules in the pituitary of the snapping turtle, Chelydra serpentina (Licht & Paphoff 1974a). Although reptiles might have two distinct hormones that appear homologous to mammalian FSH and LH, the physiology of the reptilian gonadotropins might differ markedly from those of mammals. In reptiles both these hormones appear to have the same potential physiological actions when based on gonadal activities (reviewed by Licht et al (1979a) suggested that possible differential functions under in vivo conditions may exist.

The present study is a preliminary investigation into the control of the reptilian reproductive system, especially the possibility of differential functions of FSH and LH.

6.2. Procedure
6.2.1. Collecting and housing

Mature females (SVL: 7.7 ± 0.2 cm) were collected in the Clanwilliam district (32°21' S and 19°9' E) during the winter (June).

Lizards were marked and housed as previously described in paragraphs 2.2.5.1. A non-stimulating photothermal regime (10L:14D and 20°C: 15°C) was created and the illumination period of the infra-red heating lamps (IR 240 - 250 W) was limited to 4 hours with a maximum basking temperature of 30°C. The reproductive condition of each lizard was determined beforehand through a ventro-lateral incision in the right abdominal wall (par. 2.2.5.3.).

6.2.2. Hormonal treatment

Aqueous suspensions of gonadotropins were prepared (Sodium phosphate buffer solution containing 0.1% gelatin) and administrated intraperitoneally (i.p.).

Experiments 1: Intact females (pre-vitellogenic stage) were injected i.p. daily for 10 days with either 30 µl phosphate buffer (N=4) or 10 µl pregnant mare's serum gonadotropin (PMSG) (0.2 IU. gram body mass⁻¹) or a total dose of 5 IU. per lizard) (N=5) or 10 µl human chorionic gonadotropin (HCG) (0.2 IU. gram body mass⁻¹) (N=9). Autopsy was performed as previously described (par. 2.2.3.) 24 hours after the last injection.

Experiments 2: Females containing large preovulatory ovarian follicles (11.8519 ± 6.0818 mm) following experimental vitellogenesis induction were used (see par. 2.2.5.). Lizards were daily injected intraperitoneally with PMSG (5 IU. per lizard) (N=11) and examined for ovulation during the following 24 hours (visual abdominal bulge). Additional injections followed if lizards failed to ovulate within 24 hours. Vitellogenic control lizards (N=4) were injected daily with 30 µl phosphate buffer solution and autopsied after the last experimental lizard ovulated. Autopsy procedure has been described in par. 2.2.3.
6.3. Results and discussion

Pregnant mare's serum gonadotropin (PMSG) treatment (5IU per lizard for 14 days) resulted in a highly significant increase in both ovarian and oviducal growth in the previtellogenic female Agama atra (Table 6). Similar trophic effects have been observed by various early workers on the reptilian ovary and oviduct (Mellish & Meyer 1937; Ferguson 1966). Jones (1969) reported that PMSG had a similar activity as a FSH-LH combination that he used on the lizard, Lygosoma laterale. The fact that the PMSG response was less dramatic than that elicited by purified FSH solutions, suggests an antagonizing LH effect. The failure of a large excess of LH antiserum to influence the response of FSH supports the conclusion that the action of FSH was intrinsic and not related to a trace contamination with LH (Licht & Tsui 1975b). Results obtained in Agama atra treated with PMSG are similar to results obtained in other lizards treated with purified mammalian or reptilian FSH (Jones 1968a; Licht 1970; Eyeson 1971; Licht & Hartree 1971; Licht et al 1975ab; Yaron & Widzer 1978). Significant is the report by Callard & Ziegler (1970b) that PMSG stimulation of vitellogenesis in the lizards, Dipsosaurus and Sceloponies, was growth hormone (GH) dependent whereas the response of Anolis appeared independent of GH. Hensgen et al (1980) reported that prolactin depressed growth and steroid biosynthesis of smaller ovarian follicles in the vitellogenic (FSH induced) lizard, Anolis carolinensis. As the present experiment was performed on Agama atra females with intact pituitaries the present results can neither support nor negate the idea about the requirement of GH in the vitellogenic response.

Human chorionic gonadotropin (HCG) treatment of the female Agama atra (4 IU per lizard for 14 days) resulted in a similar vitellogenic response as was evident in the PMSG treated lizards (Table 6).

Immunologically LH and HCG are related; although not identical (Goss & Lewis 1964) they show similar biological effects. Panigel (1956) reported
TABLE 6. THE EFFECT OF GONADOTROPIN THERAPY ON ORGAN MASSES\textsuperscript{a} VITELLOGENESIS, AND OVULATION IN THE PREVITELLOGENIC FEMALE AGAMA ATRA

<table>
<thead>
<tr>
<th>Treatments</th>
<th>S.V.L. (cm)</th>
<th>Ovarian Index g.100g\textsuperscript{-1}</th>
<th>Oviducal Index g.100g\textsuperscript{-1}</th>
<th>Liver Index g.100g\textsuperscript{-1}</th>
<th>Fatbody Index g.100g\textsuperscript{-1}</th>
<th>Vitello\textsuperscript{genic condition}</th>
<th>Nr OVD</th>
<th>Nr C1</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH1</td>
<td>7.93</td>
<td>9.2206</td>
<td>3.0921</td>
<td>6.2697</td>
<td>7.7945</td>
<td>XXX</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LH2</td>
<td>7.98</td>
<td>9.3273</td>
<td>1.9591</td>
<td>7.7182</td>
<td>6.8398</td>
<td>XXX</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LH3</td>
<td>7.80</td>
<td>1.3273</td>
<td>1.8038</td>
<td>4.9044</td>
<td>8.4575</td>
<td>XX</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LH4</td>
<td>7.58</td>
<td>1.0057</td>
<td>2.2279</td>
<td>4.1743</td>
<td>3.4286</td>
<td>XX</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>LH5</td>
<td>7.43</td>
<td>4.2001</td>
<td>3.7438</td>
<td>7.4892</td>
<td>6.0431</td>
<td>XXX</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>FSH1</td>
<td>7.90</td>
<td>11.2546</td>
<td>3.9415</td>
<td>13.0286</td>
<td>11.5154</td>
<td>XXX</td>
<td>9</td>
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<tr>
<td>FSH2</td>
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<td>4.1661</td>
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<td>XXX</td>
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<td>FSH3</td>
<td>7.71</td>
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<td>4.1782</td>
<td>4.2727</td>
<td>2.5009</td>
<td>XXX</td>
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<td>5</td>
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<tr>
<td>FSH4</td>
<td>7.48</td>
<td>10.7775</td>
<td>4.1667</td>
<td>7.7758</td>
<td>9.3433</td>
<td>XXX</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>CONTROL</td>
<td>7.95</td>
<td>0.5420</td>
<td>0.5939</td>
<td>5.8436</td>
<td>5.9889</td>
<td>X</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>N=4</td>
<td>0.62</td>
<td>0.0782</td>
<td>0.2323</td>
<td>0.8211</td>
<td>0.7478</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL (MARCH)</td>
<td>0.1125</td>
<td>0.2590</td>
<td>1.1100</td>
<td>1.2110</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL (JULY)</td>
<td>0.6825</td>
<td>0.8298</td>
<td>4.3706</td>
<td>2.7025</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.13307</td>
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<td>1.4796</td>
<td>0.5792</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} Presented as a corrected organ index (par. 2.2.3).

\textsuperscript{b} Condition determined according to follicle diameter.

- XXXX Preovulatory; Diameter > 5 mm
- XXX Vitellogenic; Diameter > 3 mm but < 5 mm
- XX Pre-vitellogenic; Diameter > 2 mm but < 3 mm
- X Hydration stage; Diameter < 2 mm
that HCG stimulated vitellogenesis as well as oocyte maturation in the intact lizard, Zootoca vivipara. Jones (1969) reported that HCG treatment in the lizard, Lygosoma laterale, had similar effects as purified LH in that neither increased ovarian size, but caused a moderate oviducal growth response. Similar to the present study, that of Licht (1970) suggests that both FSH and LH stimulate ovarian and oviducal growth in lizards. Although not evident in the present investigation, Licht (1970) suggests that FSH was more potent than LH. Crews & Licht (1975) suggests the possibility of important species differences between chelonians (turtles) and squamates (snakes and lizards), especially in squamates, who possibly lack dependency on LH, since this activity appears to be very low or lacking in their pituitary (Licht 1974a; Licht et al 1974 a, b). In the present study, however, both FSH and LH-like activities were evident in Agama atra.

In the female Agama atra oviducal growth was regarded as an indirect index of oestrogenic activity as suggested by Callard & Klots (1973a). Oviducal growth was stimulated in both PMSG and HCH treated Agama atra which may be indicative of steroidogenic activity. Jones et al (1975a), Lance et al (1978b, c) suggested the follicular wall of large preovulatory follicles as the major site of steroid hormone production in the squamate ovary. However, the absence of a well-defined theca interna as opposed by the presence of yolk granules and lipids in the granulosa layer in the Agama atra suggests steroidogenesis in the granulosa layer. Licht (1979b) showed that FSH is a more potent stimulator of steroidogenesis in the ovary of most reptiles, than LH. However, Lance et al (1978b) reported that the turtle, Chrysemys picta, appears to be specific to ovine FSH. The response of enzyme-dispersed turtle ovarian cells to ovine gonadotropins corroborates this suggestion (Lance et al 1978c). On the other hand, it was found that FSH and LH were both biological active in the ovaries of the snake, Natrix fasciata, in that both caused increased plasma steroid levels. It seems probable that a similar situation exists in Agama atra.
Several in vitro experiments with ovarian tissue clearly show that LH was far more potent than FSH in stimulating the uptake of radioactive precursors and resulting in increased steroid levels. (Chan & Callard 1974; Lance & Lofts 1977). Crews & Licht (1975) indicated that ovine LH was more potent than FSH, although both FSH and LH were found to stimulate in vitro progesterone production in ovarian tissue of turtles.

These apparently contradictory results are difficult to reconcile. Lance et al (1978b) suggested that the time of year at which seasonally breeding reptiles are subjected to gonadotrophic treatment, may be of critical importance in the interpretation of these results. Moreover, Licht & Tsui (1975b) suggested that the apparent inactivity of LH in lizards may reflect a relatively short half life. Daniels et al (1979) suggested that the relative high potency of some species of LH may be related in part to increased half lives.

In the present investigation it was found that chronic PMSG treatment resulted in ovulation in all the vitellogenic Agama atra females (Tables 6 & 7). The number of oviducal eggs was within the normal range, observed during the seasonal study and therefore regarded as a normal ovulation cycle. In contrast to the FSH-like activity, HCG treatment did not cause ovulation in all the vitellogenic females (Table 6).

In mammals the evidence suggests that LH may be the sole agent required to trigger normal ovulation (Schwartz 1974). Furthermore, the LH surge at the time of ovulation is usually accompanied by a FSH surge. Most information indicates that only LH was necessary, whereas the role of FSH is unclear (Espey 1978). In amphibians ovulation was also found to be highly specific for LH, whereas FSH was found to be consistently inactive. Gonadotropin specifically for ovulation in reptiles may be the opposite to that for amphibians. Licht & Tsui (1975b) reported that, although both FSH and LH induced ovulation of "ripe" follicles in Anolis carolinensis FSH clearly
### TABLE 7a. THE EFFECT OF FOLLICLE STIMULATING HORMONE (FSH) THERAPY ON THE ORGAN MASSES\(^a\), IN THE PRE-OVULATORY FEMALE, *AGAMA AGAMA* (MEANS + SD)\(^b\)

<table>
<thead>
<tr>
<th>GROUP</th>
<th>OVARIAN INDEX g.100g(^-1)</th>
<th>OVIDUCAL INDEX g.100g(^-1)</th>
<th>LIVER INDEX g.100g(^-1)</th>
<th>FATBODY INDEX g.100g(^-1)</th>
<th>NUMBER OF CORPORA LUTEA</th>
<th>PLASMA PROGESTERONE pg.0.2 ml(^{-1})</th>
<th>FOLLICLE DIAMETER mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>0.5408(^{ce})</td>
<td>0.5432(^c)</td>
<td>3.6114(^e)</td>
<td>3.4899(^{cd})</td>
<td>-</td>
<td>433.5(^{ce})</td>
<td>2.01(^{cf})</td>
</tr>
<tr>
<td>n=10</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>POST OVULATION</td>
<td>1.0495(^e)</td>
<td>2.2651(^c)</td>
<td>4.8031(^e)</td>
<td>7.6968(^c)</td>
<td>6.82</td>
<td>1550.1(^c)</td>
<td>1.92(^c)</td>
</tr>
<tr>
<td>n=11</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PRE OVULATION</td>
<td>11.8510(^c)</td>
<td>2.2377(^c)</td>
<td>4.0219(^e)</td>
<td>1.4041(^d)</td>
<td>-</td>
<td>613.2(^e)</td>
<td>8.28(^c)</td>
</tr>
<tr>
<td>n=4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^a\) Presented as corrected organ index (par. 2.2.3)

\(^b\) Student's t test probability (p) when compared with control.

\(^c\) P<0.001; Highly significant.

\(^d\) P<0.01; Significant.

\(^e\) P<0.02; Different.

\(^f\) P>0.02; Not significant.

### TABLE 7b. THE NUMBER OF FEMALES THAT OVULATED, FOLLOWING A SINGLE FSH INJECTION

<table>
<thead>
<tr>
<th>HOURS</th>
<th>4</th>
<th>8</th>
<th>12</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=11</td>
<td>Animals with oviducal eggs.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>CONTROL</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
appeared to be the more potent in this regard. Moreover, immunoabsorption of LH contamination had no effect on the ovulation activities, underlining the intrinsic FSH activity. Similar results were reported by Licht & Hartree (1971).

Unfortunately no experimental work has been done to ascertain whether LH possesses intrinsic ovulatory activity. Seasonal circulating levels of gonadotropins (FSH and LH) were studied in the turtle, *Chelonia mydas*, and an LH and concomitant progesterone surge during the ovulation cycle were clearly indicated. Significantly, the FSH plasma concentration, only increased at the time of oviposition. Licht (1979b) therefore concluded that the two gonadotropins (FSH & LH) may well play separate roles in the regulation of certain aspects of ovarian function. In contrast to the available data from *in vitro* physiological studies of reptiles, it now appears that LH rather than FSH is the hormone responsible for the major progesterone surge and probably for the induction of ovulation in *Chelonia mydas*. Although the FSH-like PMSG did not cause early oviposition in the *Agama atra* females, Licht & Crews (1975a) observed that FSH induced oviposition in female lizards. In the present investigation the PMSG and HCG treatment was terminated after 16 days and PMSG treatment to vitellogenic females after the presence of oviducal eggs became evident.

It was noteworthy that large vitellogenic ovarian follicles were present together with apparent functional corpora lutea and oviducal eggs in all gonadotropin-treated *Agama atra* (Table 6). The precise origin of the increased progesterone levels during this stage is unclear, although the corpora lutea may be the most likely production area. The role of progesterone as an antigonadal hormone is questioned. Yaron & Widzler (1978) suggested that progesterone probably inhibited the action of gonadotropin on the follicular wall with the result that the yolk precursor uptake by the oocyte it inhibited. However, ovarian growth observed in the presence of high progesterone levels in the *Agama atra* female does not
con corroborate this suggestion.

Conclusions

1. PMSG and HCG therapy caused significant increases in both ovarian and oviducal growth in Agama atra.

2. The present investigation did not corroborate the general suggestion that FSH is more potent in stimulating ovarian growth in squamates.

3. It was evident that FSH was more potent in stimulating the induction of ovulation.

4. High progesterone levels did not inhibit oocyte accumulation of yolk precursors and therefore did not influence gonadotropin activity on the follicular wall.

5. Much more research is needed to elucidate the structure and function of the squamate gonadotropins.
CHAPTER 7

THE EFFECT OF NEUROHYPOPHYSIAL HORMONES ON THE OVIDUCT

7.1. Introduction

Oviposition has been induced in various gravid female lizards by injecting neurohypophysial factors (Panigel 1956; Munsick et al. 1960; La Pointe 1964, 1977; Heller 1972; Callard & Hirsch 1976; Ewert & Zegler 1978; Guillette 1979). Furthermore, Arginine vasotocin was found to be more potent than oxytocin in the ability to elicit oviducal contractions in the viviparous lizard, Klauherina riversiana (La Pointe 1969). Lemus et al (1970) reported a greater response to oxytocin, especially oviducts obtained during late gestation. It is significant that arginine-vasotocin and mesotocin, but not oxytocin, have been isolated from Iguana iguana (Archer et al. 1972).

The present investigation concerns the sensitivity of the intact oviduct of pregnant Agama atra to arginine-vasotocin and oxytocin. This was only undertaken as a preliminary investigation to further study concerning the function of progesterone in Agama atra.

7.2. Procedure

7.2.1. Collecting and housing

Similar to par. 2.3.1, the lizards were exposed to a stimulating photothermal regime (14L:10D and 35°C) ambient temperature. The degree of pregnancy was estimated after abdominal palpation.

7.2.2. Hormonal treatment and experimental procedure

Lizards were randomly partitioned into three groups:

Group 1: Single intraperitoneal (i.p.) injection of 0.1ml of 0.75% physiological saline.

Group 2: Single intraperitoneal injection of 4µl arginine vasotocin (Sigma) (0.2 µg per lizard).
Group 3: Single intraperitoneal injection of 4 μl oxytocin (synthetic) 
(10IU.ml⁻¹) (Ciba-Geigy).

Several dependent measures of oviposition were obtained including:

i. initial contraction latency (the time from injection to first visual contraction).

ii. oviposition latency (the time from injection to first birth).

iii. interbirth duration (the period between births).

The behavioural response of the females after receiving the i.p. injection was observed. Maximum experimental duration was taken as 360 minutes after the i.p. injection.

7.3. Results and Discussion

Neither oviducal contractions nor oviposition were observed in saline or arginine-vasotocin treated females (table 8), but one female responded to mammalian oxytocin. The lizard which responded to mammalian oxytocin showed initial contractions 21 minutes after the injection and oviposition followed 60 seconds later. The mean duration between the births was 6.74 ± 0.94 minutes and the behavioural patterns described by La Pointe (1964) and Guillette (1979) were not observed. Noteworthy was that all females laid eggs naturally 7-14 days after the experiment had been performed.

The negative results obtained in the female Agama atra are difficult to explain because all the lizards were in a late gestation stage. Whether ovarian steroids influenced the contractility of the Agama atra oviduct is not known. Callard & Hirsch (1976) reported that oestradiol enhanced the amplitude of arginine-vasotocin induced oviducal contractions in vitro in the turtle, Chrysemis picta, while progesterone had no effect on the amplitude but reduced the duration of contractions. In contrast to these findings La Pointe (1969)
<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>N</th>
<th>SVL (cm)</th>
<th>INITIAL CONT. LATENCY (MIN: SEC)</th>
<th>OVIPOSITION LATENCY (MIN: SEC)</th>
<th>DURATION BETWEEN BIRTHS (MIN: SEC)</th>
<th>NUMBER OF EGGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>SALINE</td>
<td>2</td>
<td>7.25</td>
<td>360 : 00</td>
<td>360 : 00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.75%</td>
<td></td>
<td></td>
<td>0.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARGinine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VASOTOCIN 0.2µg</td>
<td>4</td>
<td>7.30</td>
<td>360 : 00</td>
<td>360 : 00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OXYTOCIN 0.2µg</td>
<td>1</td>
<td>7.64</td>
<td>21 : 00</td>
<td>22 : 00</td>
<td>6.74 ± 0.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>3</td>
<td></td>
<td>360 : 00</td>
<td>360 : 00</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* AVT = Arginine - vasotocin
reported that steroids did not influence oviducal contractions in the viviparous lizard, *Klauberina riversiana*. Guillette (1979) proposed the hypothesis that the oviduct possesses an intrinsic frequency of contractility and that the strength may be influenced by hormonal conditions during pregnancy. Heller (1972) and Callard & Hirsch (1976) pointed out that the sensitivity of the reptilian oviduct to neurohypophysial hormones may be dependent on the physiological state of the tissue. The dose-response relationship used in the present investigation may be a critical factor for each species and therefore require further investigation. The fact that one lizard responded to oxytocin treatment, however, suggests that some response exists and that further investigation using a different experimental procedure is needed.

7.4. Conclusion

Oviposition could not be induced by using arginine-vasotocin or oxytocin in pregnant *Agama atra*, which may be the result of steroid influences at the time. Seasonal progesterone concentrations indicated relatively high plasma progesterone during pregnancy and therefore further investigation is needed on the possible influence of progesterone on the oviducal contraction of the oviparous lizard.
SUMMARY

Owing to the lack of information on the reproductive cycles of South African lizards, the morphological and physiological changes during the annual reproductive cycle of the female oviparous lizard, *Agama atra*, were described.

The present study was undertaken in the Fynbos Biome (Coastal Macchia) which is currently receiving increased attention because of the uniqueness and zoogeographic importance of this region.

Vitellogenic ovarian hypertrophy during September marked the onset of the annual breeding season. At least two clutches were subsequently produced and no vitellogenic cycles were evident after February. The spring and autumnal equinox as well as change in air temperature correlated with the onset and termination of the breeding cycle. Photothermal regimes, simulating summer conditions induced vitellogenesis in females collected during winter months.

Oogonial proliferation, oogenesis and folliculogenesis in the female were found to be consistent with the general squamate pattern. The presence of the large flaskshaped pyriform cells and their intercellular connections with the ooplasm, during active vitellogenesis stressed the need for more insight into the role of these cells. Hypertrophy of the granulosa marked the post-ovulatory follicles, which may indicate a functional significance while the eggs are in the oviduct. Two types of atretic follicles were observed, occurring throughout the ovarian cycle.

The fatbody showed a typical inverse correlation with ovarian hypertrophy, suggesting fatbody utilization for the production of the first clutch. A decrease in total plasma cholesterol correlated with ovarian hypertrophy.
which may be indicative of increased ovarian uptake during vitellogenesis. Likewise, the total plasma protein concentration decreased during vitellogenesis. However, during this period a marked increased in protein fraction II (similar mobility as human beta-globulin) occurred but a concomitant decrease in the foremost migrating plasma protein fractions may have disguised significant seasonal changes in the total plasma proteins.

Total plasma calcium concentration increased significantly during vitellogenesis. No special calcium stores could be detected, thereby suggesting that adequate calcium is available through the diet and possibly bone resorption for the production of the yolk precursor, vitellogenin. A post-ovulatory progesterone surge occurred in the presence of well developed corpora lutea. Noteworthy was that the onset of a second vitellogenesis cycle was observed in the presence of relative high progesterone levels which may question the postulated anti-gonadal effect of progesterone. The oviducal index showed a high correlation with ovarian development, indicating steroidogenic activity (oestrogenic).

Oestrogen and oestrogen plus progesterone treatment in the male Agama atra resulted in increased plasma vitellogenesis parameters, whereas progesterone alone had no effect. Both PMSG and HCG treatment induced vitellogenesis in females containing quiescent ovaries, although PMSG was found to be more potent in inducing ovulation. Neither arginine-vasotocin nor oxytocin could induce early oviposition in late gestation stage females.

In general this study indicated that much more elaborate investigations are needed to demarcate the breeding season and to investigate the seasonal changes in the blood parameters associated with vitellogenesis in oviparous lizards.
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* Not seen in the original.