# REPRODUCTIVE BIOLOGY OF *CHAMAELEO PUMILUS PUMILUS* WITH SPECIAL REFERENCE TO THE ROLE OF THE CORPUS LUTEUM AND PROGESTERONE*

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## ABSTRACT

The morphology of the female reproductive tract and of the ovaries of the ovoviviparous *C. pumilus* was studied. Sperm storage organs were found in the vaginal region and changes in the histological appearance of the corpus luteum indicated a cyclic function similar to that found in mammals. Studies with $^{14}C$ labelled leucine suggested that amino acid transfer occurred between the mother and the embryos. A radio-immuno assay of the progesterone content of the corpus luteum showed that the corpus luteum contains 56,8 μg progesterone/g of fresh luteal tissue and that the mean plasma levels of progesterone increased from 945,6 pg/ml in non-gravid to 2296,0 pg/ml in gravid females. There also appears to be a post-ovulatory surge of progesterone (4946,0 pg/ml). Oestrogens were found to stimulate the oviducal mucosa while progesterone acted synergistically with oestrogen. Oestrogens also stimulated the production of serum protein fractions. Progesterone caused yolk regression and thus prevented the onset of the next follicular phase. Studies involving ovariectomy and progesterone replacement, indicated that progesterone and the corpus luteum are essential for the maintenance of gestation. It was concluded that several features of the reproductive biology of *C. pumilus* can be considered to be distinctly mammalian and that these features are of considerable evolutionary importance.

## INTRODUCTION

Ovoviviparity in reptiles is a phenomenon which has received surprisingly scant attention from physiologists. This is regrettable in view of the important evolutionary implications involved. Moreover, little work has been done on the function of progesterone in non-mammalian vertebrates and the role played by this hormone during gestation is still obscure. The weight of evidence to date suggests that the corpus luteum is not essential to the maintenance of gestation in reptiles (Clausen 1940; Bragdon 1952; Callard *et al.* 1972) but there is no certainty on this issue. That the corpora lutea of reptiles are able to synthesize progesterone is well known (Callard & Leathem 1963), but the precise levels of this hormone in the corpus luteum in the natural state, and of the circulating progesterone levels in the blood, are still unknown. Also the evidence on the effects of progesterone and oestrogens in reptiles is conflicting.

The general ecology of *Chamaeleo pumilus pumilus* (*Microsauria pumilus*) together with data on its reproduction, has been well documented by Burrage (1973), but the specific physiological roles of the corpus luteum and progesterone were not studied.

The purpose of the present investigation was therefore to examine the general reproductive biology of *C. pumilus* by studying the morphology of the reproductive tract and the transfer of nutrients between the mother and the embryos and more specifically to elucidate the precise role of progesterone in the gravid female.

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## PROCEDURE

The ovoviviparous lizard, *Chamaeleo pumilus pumilus*, was selected for this study because this species was readily available in the Stellenbosch district. Individuals were collected when they were needed and when an experiment required that they should be maintained in the laboratory, they were allowed to roam freely on a small tree in a laboratory maintained at room temperature. When animals were grouped for experimentation, they were housed in separate cages 30 × 30 × 15 cm. They were fed on the larvae of *Tenebrio molitor* and received water *ad libitum*.

### *Morphology of the female reproductive tract*

The oviduct of a female (follicles in the yolking stage) was excised and fixed in 10% formol saline for 24 hours and then embedded in paraffin (melting point 54°C). Serial sections (10 μ) were then prepared and stained with azocarmine and azan.

### *The transfer of maternal nutrients to the embryos*

To determine whether nutrient transfer occurs between the embryos and the mother, six gravid female chamaeleons were selected and placed in separate cages. A single injection of $^{14}C$ labelled leucine (37 913 cpm), dissolved in redistilled water, was administered subcutaneously to each female. The animals were kept for one week, after which they were killed by decapitation and the livers and embryos removed and weighed. The embryos were removed with particular care to ensure that they did not become contaminated with maternal tissue fluids containing radio-active material. The livers and embryos were then homogenized separately and quantitatively transferred into a counting vial. Scintillation fluid (10 ml) consisting of 100 ml BIO-SOLV-Solubiliser (BBS-3), and 8 g TLA Floure, made up to one litre with Beckman scintillation toluene, was added to each vial and counted in a Beckman model (LS-133) scintillation counter.

### *The histology of the corpus luteum at various stages of gestation*

In order to study the histology of the ovary, five females were collected during each stage of gestation (ranging from early pregnancy to one month *post partum*). They were killed by decapitation, the ovaries were then excised, fixed in 70% ethyl alcohol, embedded in paraffin and sectioned at 5 μ. The sections were stained with azocarmine and azan.

### *Progesterone levels in the corpus luteum*

Luteal tissue with a total weight of 56 mg was collected from the ovaries of ten gravid chamaeleons. Only fully formed corpora lutea were used. The corpora lutea thus obtained were homogenized and an ethyl alcohol extract prepared. The progesterone content of the extract was then determined by means of a radio-immuno assay using dextran-coated charcoal. All the reagents including the antibody, were supplied by Biolab, Brussels, Belgium. All samples were counted in a Beckman scintillation counter model LS-133.

### *Plasma levels of progesterone in relation to the reproductive status*

Throughout the year female chamaeleons were collected and blood samples taken after decapitation. Blood samples were collected from five chamaeleons in each of the following reproductive stages: yolking stage, post-ovulatory stage and gravid stage. In addition blood samples were taken from three males and the blood from three non-yolking non-gravid females was pooled to make up one sample.

All the samples were centrifuged at 2 500 rpm in a clinical centrifuge for 15 minutes and the plasma stored at $-15°C$ for later analysis. For assay purposes 250 $\mu\ell$ of plasma from each sample was extracted with ether three times. All mixing was done with a whorly-mixer, the plasma was then snap-frozen by inserting the test tubes in a mixture of acetone and dry ice and the ether layer decanted. A radio-immuno assay, as previously described, was carried out on the ether extract to determine the progesterone content of the plasma.

### *Physiological effects of progesterone and oestrogen upon the juvenile reproductive tract*

Only juvenile females, weighing less than 5 g were used in this study; the weight range was 2,15–4,65 g. Two animals were allotted to each treatment and they were housed in separate cages. The following treatments were employed:

1. Control group which received daily injections of ethyl alcohol only
2. Daily injections of 5 mg 17-$\beta$ oestradiol per 100 g body weight
3. Daily injections of 5 mg progesterone per 100 g body weight
4. Daily injections of 5 mg 17-$\beta$ oestradiol plus 5 mg progesterone per 100 g. body weight.

The hormones were administered in ethyl alcohol, as the administration of hormones dissolved in cotton-seed oil had a toxic effect on the animals. In order to inject as little alcohol as possible, the solutions were saturated. The injections were administered for seven days and one day after the last injection the lizards were killed, the oviducts were excised, fixed in 70 % ethyl alcohol and after routine embedding in paraffin and sectioning at 8 $\mu$, the sections were stained with azocarmine and azan.

### *Vitellogenesis and the effect of oestrogen and progesterone on serum proteins*

To study the effect of ovarian hormones on the serum protein fractions, twelve juvenile female chamaeleons and sixteen males were collected. The females were grouped into four groups of three and the males into four groups of four. They were housed in separate cages. One group of each sex acted as a control and received daily injections of ethyl alcohol only. The other groups received the following treatments:

1. Daily injections of 5 mg 17-$\beta$ oestradiol per 100 g body weight
2. Daily injections of 5 mg progesterone per 100 g body weight
3. Daily injections of 5 mg 17-$\beta$ oestradiol plus 5 mg progesterone per 100 g body weight.

The injections were administered for seven days and one day after the last injection the lizards were killed by decapitation and a blood sample was taken by means of a capillary tube and allowed to clot. The blood was then centrifuged at 2 500 rpm in a clinical centrifuge and the serum stored at $-15°C$ for later analysis. Electrophoresis of the serum was carried out by means of a Beckman micro-zone electrophoresis system using cellulose acetate membranes.

In order to determine when vitellogenesis took place in the adult, plasma protein analyses were performed on adult females in various stages of the reproductive cycle. Five females each in the non-gravid, non-gravid yolking and gravid stages, were selected and their plasma was subjected to electrophoresis on cellulose acetate membranes.

### *The effect of progesterone on yolked follicles*

Thirteen females in the yolking stage were selected. The animals were cooled until their cloacal temperature approached 0°C, and then a small median incision was made in the abdominal wall. The follicular diameter was measured with dividers and the average taken to represent the follicular diameter of the yolking stage in question (Table 4). The incision was closed with 4–0 thickness sterile nylon and the animals were then kept in separate cages for the duration of the experiment. During this time they fed and behaved normally and the laporotomy did not seem to affect them adversely. Seven were injected daily with 0,05 mg progesterone and six received a daily dose of the solvent (ethyl alcohol). After two weeks they were killed and the follicular diameter and degree of yolking was again measured.

### *Effect of ovariectomy and progesterone replacement therapy upon gestation*

Eighteen gravid chamaeleons were collected. Twelve were ovariectomized and sham operations were performed on the remaining six.

The animals were anaesthetized by cooling them to ± 0°C and the ovariectomies were carried out under sterile conditions. A median incision was made in the abdominal wall and the two oviducts containing the embryos were moved to the sides. The ovaries were then excised and the incision closed with 4–0 sterile nylon. Six of the ovariectomized group received 0,2025 mg progesterone every third day and the remaining six received 4 $\mu\ell$ ethyl alcohol (which was the same volume of solvent injected into the experimental animals) every third day. The chamaeleons on whom sham operations had been performed received no treatment.

During the first few days after the operation, the chamaeleons had to be treated with great care as they were prone to haemorrhage from the ovarian artery, but they recovered rapidly if they survived the first two days. The duration of the experiment was two weeks in view of practical considerations.

## RESULTS AND DISCUSSION

### MORPHOLOGY OF THE FEMALE REPRODUCTIVE TRACT

The morphology of the reptilian oviduct has been studied on a comparative basis by Cuellar (1966). He noted that sperm storage organs were common among iguanids and that in the majority of cases they were confined to the vaginal region. Saint Girons (1962) noted sperm receptacles in the vaginal region of *Chamaeleo lateralis* and Yaron (1972) indicated similar structures in the viviparous lizard *Xantusia vigilis.*

The oviducal histology of *Chamaeleo pumilus* has not previously been studied and sperm storage organs have thus not been definitely indicated in this species. Atstatt (1953) presumed that such structures were present in *C. pumilus* because he observed that an isolated female went through two to three gestation periods without mating.

### *Gross anatomy*

The oviduct is divided into an anterior infundibulum and Fallopian tube, a middle, uterine section and a posterior vagina which opens into the cloaca.

### *Infundibulum*

The infundibulum is the widest part of the oviduct. It is surrounded by a very thin circular muscle layer but a longitudinal muscle layer is absent. The mucosa is strongly folded and unciliated and the height of the villi is greater medially than laterally where the epithelium flattens to form a uniform layer. The mean height of the villi in this region is only 84,5 μ. The vascularity of the infundibulum is poor and there appear to be no blood vessels in the villi.

### *Fallopian tube*

The Fallopian tube is surrounded by a circular muscle layer. The mucosa is again folded to form villi, the height of which decreases from the anterior end to the middle and increases again from the middle to the posterior end. The villi are poorly vascularized in the anterior reaches of the tube but the vascularity increases at the uterine end where occasional blood vessels appear in the tips of the villi. The epithelium is not ciliated and it would appear as if the ovum is forced into the tube by muscular contractions only.

Tubal glands occur beneath the epithelium. These are surrounded by peritoneum impregnated with a strong black pigmentation. This pigmentation, probably melanin, is common to the entire oviduct with the exception of the posterior section of the vagina. Tubal glands become more numerous towards the middle of the Fallopian tube. Posteriorly, the Fallopian tube is also surrounded by a longitudinal muscle layer which is at first a smooth layer on the outside of the circular muscle layer, but this becomes convoluted in the extreme posterior end.

### *Uterus*

The uterus is surrounded by an inner circular muscle layer and an outer convoluted longitudinal muscle layer (Figure 5). In contrast to the Fallopian tube and vagina, the uterus is extremely glandular and many subepithelial glands occur. The epithelium is not ciliated. The vascularity of the uterus is far greater than that of the other segments of the oviduct, and each villus possesses a blood vessel in the tip, projecting into the lumen (Figure 6).

The blood vessels in the tips of the villi lie in close proximity to the surface blood vessels of the yolk sac. Gas exchange in all probability takes place by means of these blood vessels and the possibility of nutrient transfer is not excluded.

### *Vagina*

Posteriorly the vagina enters the cloaca, and runs in a large ventrally situated fold of the cloacal mucosa.

In this region it is surrounded by a firm circular muscle layer, while longitudinal muscles are absent. The vaginal epithelium is arranged in folds and is very strongly ciliated (Figure 7). The number of mucosal folds increases from posterior to anterior; there is a minimum of three folds posteriorly and a maximum of thirty-three anteriorly. The average height of these folds is 150,7 μ. The increase in fold number is a result of bifurcation of existing folds.

Posteriorly to anteriorly there is a decrease in ciliation, and in the anterior region the cilia are arranged in clusters and no longer form a uniform lining to the vaginal epithelium. The vagina is poorly vascularized but the vascularity increases anteriorly.

The seminal receptacle consists of a single layer of ciliated cells. Posteriorly seminal receptacles are not numerous but a few occur in the mid-vaginal region (Figure 8). In the anterior portion of the vagina, seminal receptacles are more numerous and they sometimes appear in groups or seminal receptacle complexes.

In conclusion it may be said that the gross anatomy of the oviduct of *C. pumilus* is similar to that of the iguanids studied by Cuellar (1966). The seminal receptacles are very similar to those found in the mid-vaginal region of *Sator grandaevus* and the general histological appearance of the oviduct of *Chamaeleo lateralis* (Saint Girons 1962) is also similar to that of *C. pumilus*.

## THE TRANSFER OF NUTRIENTS TO THE EMBRYOS

In view of the close contact between uterine blood vessels and embryonic blood vessels, the possibility of a nutrient link between mother and embryo was examined.

A single injection of $^{14}C$ labelled leucine (37 963 cpm) resulted in the absorption values shown in Table 1. These values suggest that small amounts of the amino acid leucine are transferred from the maternal circulation across the oviduct to the embryonic circulation. The metabolic pathways involved, however, are too complex to accept this facile conclusion. For example the injected amino acid may have undergone de-amination or decarboxylation and the transfer that was measured may only have involved a simpler carbon fragment. Nevertheless, the results obtained are encouraging and further work employing a greater variety of radio-active tracers is indicated.

TABLE 1

QUANTITIES OF RADIO-ACTIVE LEUCINE ($^{14}C$) ABSORBED IN CPM BY LIVER AND EMBRYOS OF *C. pumilus*

| *Number of animals used* | *Liver weights (mg) (X ± S.D.)* | *Total cpm per liver (X ± S.D.)* | *Number of embryos per gravid female (X ± S.D.)* | *Total embryo weight per gravid female (mg) (X ± S.D.)* | *Total cpm per embryo batch (X ± S.D.)* | *Background cpm* |
|---|---|---|---|---|---|---|
| 6 | 268,0 ± 107,7 | 366,5 ± 97,2 | 13 ± 2,7 | 3410,6±554,6 | 155,9 ± 16 | 5,2 |

## THE HISTOLOGY OF THE CORPUS LUTEUM AT VARIOUS STAGES OF GESTATION

Corpora lutea have been described in many species of ovoviviparous and viviparous lizards (Weekes 1934; Miller 1948) and snakes (Bragdon 1952; Betz 1963). Weekes (1934) found that the corpus luteum of viviparous lizards exists for approximately three and one-half months and that degeneration commences while the animals are still pregnant. In the garter snake Bragdon (1952) found that there is little difference between the corpus luteum at term and at mid-gestation but six weeks after parturition there are marked signs of degeneration. In the diamond-backed water snake, *Natrix rhombifera*, the corpus luteum also retains its structural integrity to term (Betz 1963).

### *Ovary of non-gravid C. pumilus females*

At this stage the ovary is a small organ approximately 7 mm in length and 3–4 mm wide. It consists of a median strip of connective tissue to which a varying number of non-yolked Graafian follicles are attached (5–17 in the eight specimens used during the current examination). No signs of any corpora lutea or corpora albicantia were observed. In cross section the Graafian follicles appear as circular structures filled with liquor folliculi. The ova are usually situated peripherally in the follicle, close to the theca interna.

Just before ovulation the follicles become yolked and they increase greatly in size. The diameter of each individual follicle may increase from 0,75 mm before yolking, to 7,5 mm after yolking. After ovulation the opening for the ovum is visible as a depression on the follicles which may now be regarded as corpora haemorrhagica.

### *Corpus luteum during early gestation*

The corpora lutea are fully formed at this stage and are surrounded by a firm theca from which connective tissue and theca-cell strands converge towards the centre of the corpus luteum. The lutein cells are reasonably compacted but this compactness declines towards the centre of the corpus luteum. In the centre the intercellular spaces are larger than in the case of the peripheral cells. The cells are also arranged in a random fashion and are not uniformly orientated (Figure 9). The follicles were small at this stage and it is possible that the corpus luteum retards follicular growth in this species.

### *Corpus luteum during mid-gestation*

At this stage the corpora lutea are well developed and the lutein cells are more compacted and slightly larger than during early gestation. The connective tissue strands which converge towards the centre of the corpus luteum are more distinct than in the previous stage and in some cases link up with a central cavity (Figure 10). The cavity is spherical and does not seem to be lined by any distinct membrane. It can also be considered a common feature in the corpora lutea of this species.

### *Corpus luteum during late gestation*

The corpora lutea at this stage are still compacted structures but the lutein cells are smaller than in the previous stages (Figure 11). This decrease in cell size results in the cells not being closely aligned. The increase in intercellular space size is especially apparent in tangential section through the corpus luteum.

*Corpus luteum one hour after parturition*

In sections made through the centre of the corpus luteum, the organization of the lutein cells shows marked signs of deterioration (Figure 12). Large cavities occupy the centre of the corpus luteum and the lutein cells do not adhere to each other. This is probably as a result of lipid infiltration. In tangential sections through the corpus luteum the deterioration is not as marked but the intercellular spaces are larger (Figure 13).

*Corpus luteum one month after parturition*

The corpora lutea at this stage are entirely invaded by connective tissue and have deteriorated to such an extent that they may be regarded as corpora albicantia. A thin layer of theca cells surrounds each corpus albicans but the number of lutein cells present has diminished to such an extent that they appear as isolated cells surrounded by connective tissue (Figure 14).

It is clear therefore that in *C. pumilus* the histological appearance of the corpus luteum changes as pregnancy progresses. The lutein cells are the most compact during mid-gestation. The first signs of deterioration are apparent during late gestation and after parturition this deterioration is very rapid. It takes approximately six weeks for the corpora albicantia to disappear. From a histological point of view it would thus seem as if the corpus luteum in *C. pumilus* displays a cyclic function as is the case in mammals and that it probably plays an important part in the maintenance of gestation.

TABLE 2

MAXIMUM CONCENTRATIONS OF PROGESTERONE IN LUTEAL TISSUE OF VARIOUS SPECIES

| *Species* | *Reference* | *Progesterone µg/g luteal tissue* |
|---|---|---|
| Cow (*Bos taurus*) | Gomes & Erb (1965) | 73,2 |
| Chamaeleon (*Chamaeleo pumilus*) | Present study | 56,8 |
| Sheep (*Ovis aries*) | Deane *et al* (1966) | 50,0 |
| Human (*Homo sapiens*) | Zander *et al* (1958) | 49,8 |
| Guinea pig (*Cavia porcellus*) | Rowlands & Short (1959) | 37,6 |
| Seal (*Phoca vitulina*) | Short (1958) | 71,0 |
| African elephant (*Loxodonta africana*) | Smith *et al* (1969) | 0,18 |

## PROGESTERONE CONTENT OF THE CORPUS LUTEUM

It is known that the corpora lutea of reptiles are able to synthesize progesterone (Callard & Leathem 1963) but an accurate quantitative determination of the progesterone content of corpora lutea in reptiles has not been carried out. The concentration of progesterone in the corpus luteum is indicative of the activity of this gland and also of its consequent importance as an endocrine gland.

The radio-immuno assay showed that progesterone is present in the corpora lutea of *C. pumilus* at a concentration of 56,8 μg/g of fresh luteal tissue. This value is surprisingly high and falls well into the range of progesterone values known for mammals (Table 2). It would therefore appear as if progesterone plays an important part in the reproductive biology of *C. pumilus*. It should, however, be borne in mind that the adrenals of reptiles are also able to synthesize progesterone as was shown for the cobra *Naja naja* (Huang *et al.* 1969), but the high values of progesterone found in the corpora lutea of *C. pumilus* suggest that the corpus luteum is the main source of progesterone in this species. A study of the plasma levels of progesterone during various reproductive stages would, however, lend greater credance to this idea.

## PLASMA LEVELS OF PROGESTERONE IN RELATION TO THE REPRODUCTIVE STATUS

Identification of progesterone in the peripheral blood is an essential step in attributing a specific function or functions to this hormone in any species. Moreover, changing plasma levels of progesterone in the course of the reproductive cycle would be evidence of a more conclusive nature.

Progestins have been identified in the plasma of the viviparous lizard, *Tiliqua rugosa* (Gray), (Bourne & Seamark 1972). These authors, however, employed a competitive protein-binding technique to assay progesterone which, in view of its limited specificity, cannot be considered conclusive. The present study represents the first time that the highly specific radio-immuno assay has been employed to determine the progesterone levels at various stages in the reproductive cycle of an ovoviviparous reptile.

The results of this assay showed that the plasma levels of progesterone increased from 945,6 ± 713,86 pg/ml during the yolking stage to 4 946 ± 3 902,19 pg/ml after ovulation. During pregnancy the values were 2 296 ± 338,94 pg/ml (Figure 1). The increase in progesterone from the yolking stage to the post-ovulatory stage is significant ($P < 0,10$) and the increase from the yolking stage to the pregnant stage is highly significant ($P < 0,01$).

The average progesterone value in the plasma of three males was 632 pg/ml. The plasma of three non-gravid, non-yolked females was pooled, and found to contain 864 pg/ml. These values clearly show that there is a post-ovulatory surge of progesterone and that during pregnancy the progesterone levels are kept uniformly high. The high deviation from the mean, in the post-ovulatory stage, can be explained, as some of these samples were taken from animals with fully formed corpora lutea while others were obtained from animals still possessing corpora haemorrhagica.

The progesterone levels in the male are, as expected, very low and progesterone is probably present in this case as an intermediate metabolite. The results obtained in this study are not at all in accordance with those obtained by Bourne & Seamark (1972). They found the mean progestin values in adult males of *Tiliqua rugosa* to be 15,9 ng/ml. This concentration is higher than the progesterone concentration in the human during early pregnancy. It is possible that the competitive protein-binding technique used in determining the progesterone concentrations was not specific enough and that other steroids were also measured. This may also explain the very high standard deviation which they obtained.

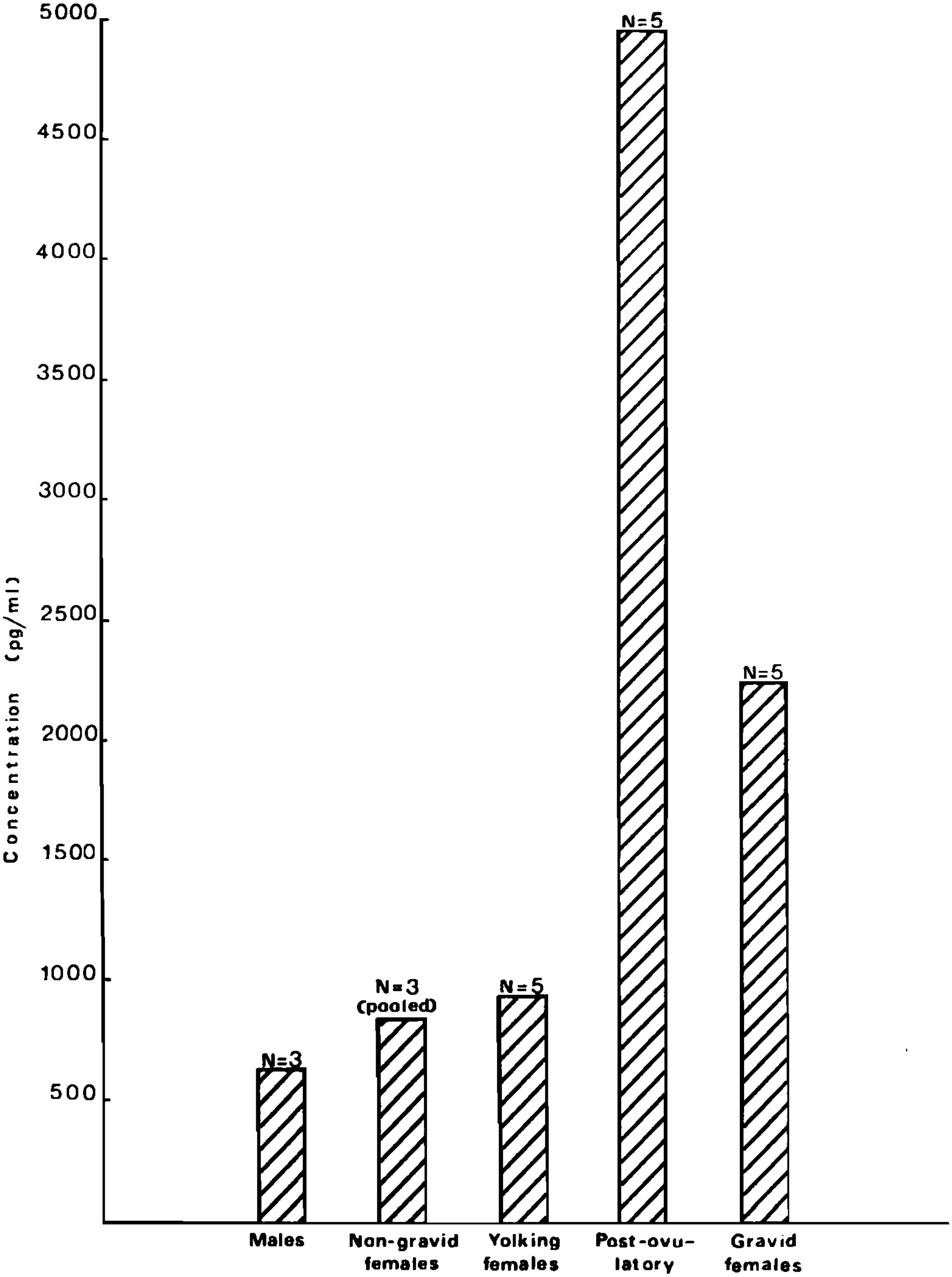

FIGURE 1

The plasma levels of progesterone in *Chamaeleo pumilus* during various phases of the reproductive cycle.

It would seem therefore that progesterone, in view of its cyclic secretion, is involved in the maintenance of gestation and also in the general reproductive biology of *C. pumilus*.

## PHYSIOLOGICAL EFFECTS OF PROGESTERONE AND OESTROGEN

### *Upon the juvenile reproductive tract*

In general it is accepted that the differentiation of the Müllerian duct in reptiles occurs during the breeding season and that ovarian hormones are the initiators of this process (Yaron 1972, Callard & Leathem 1970). Moreover, oestrogens are commonly believed to be the steroids responsible for the maturation of the oviduct, as these steroids increase the oviducal weight and bring about the differentiation of the oviducal mucosa (Prasad & Sanyal 1969).

In the present study the effect of Pregnenolone, 17*β* oestradiol and a combination of the two hormones on the oviducal mucosa of juvenile chamaeleons was studied.

In control animals the epithelium of the infundibulum was found to be columnar and distinctive villi were absent. In animals treated with progesterone the epithelium was also columnar and spread as a uniform layer with occasional very small villi. It would thus appear that progesterone had little or no effect on the proliferation of the infundibular epithelium. In contrast, infundibular epithelium of juveniles treated with oestrogen and with a combination of oestrogen and progesterone, showed definite, small villi, the height of the villi being greater in the latter treatment.

It seems thus as if oestrogens cause proliferation of the infundibular epithelium and that progesterone enhances this effect, indicating a synergism between the two hormones. The effects of the hormones on the uterine section of the oviduct were similar to those observed in the infundibular segments.

### *Vitellogenesis and the effect of oestrogen and progesterone on serum proteins*

Very little is known about vitellogenesis in reptiles during the normal reproductive cycle. Gerstle & Callard (1972) found that the *β* fraction increased significantly in *Diplosaurus dorsalis* during vitellogenesis. The effect of steroid hormones on the serum proteins of reptiles has been more extensively studied and Clark (1967) and Gerstle & Callard (1972) found that oestrogens significantly increased the total proteins. Rao & David (1967) showed that anabolic androgens had a significant effect on the serum protein concentrations of the lizard, *Uromastix hardwickii*, the effect being mainly on the *a* globulins. There is, however, very little information on the effect of oestrogens, specifically 17 *β* oestradiol and progesterone on the serum protein constituents of reptiles. In birds, oestrogens cause an increase in the *γ* globulins, as a result of an increase in phosvitin, a phospholipoprotein. Yolk and plasma phosvitin have a very similar composition and this phospholipoprotein is thus confined to oviparous animals (Butler 1971).

The results obtained in the present investigation showed that the serum from control animals included albumin, *a* 1, *a* 2 and *γ* globulins. The *γ* globulins could not be clearly separated into *γ* 1 and *γ* 2 (Figure 2).

The injection of oestrogens brought about a marked change in the electrophoretic pattern of the *γ* golubulins. The *γ* 1 and *γ* 2 globulins could be clearly distinguished and in all cases the percentage $\gamma$ 1 was greater than that of $\gamma$ 2 (Figure 3). Oestrogens thus have an effect on the production of the $\gamma$ globulins which are also the main constituents of the yolk.

It can be presumed that phosvitin production probably plays an important part in yolk formation and that oestrogens cause the synthesis of this phospholipoprotein by the liver. The above result is substantiated by an electrophoretogram of yolk as it appears in the yolked follicles of the chamaeleon ovary (Figure 4). Except for a slight trace of albumin, the $\gamma$ globulins are the only protein constituents of the yolk when separated by cellulose acetate electrophoresis.

Administering progesterone also affected the electrophoretic separation of the $\gamma$ globulins, but the effect was not as marked as that produced by oestrogen. It appears, however, that the $\gamma$ 2 globulin is increased in all the animals injected with progesterone.

When both oestrogen and progesterone were injected the electrophoretic trace was similar to when only oestrogen was injected. This suggests that oestrogen has a more profound effect on the $\gamma$ globulins than does progesterone. Clearly further study is necessary to illuminate this problem, but it is probably safe to assume that oestrogens have a similar effect on the production of proteins as is the case in birds.

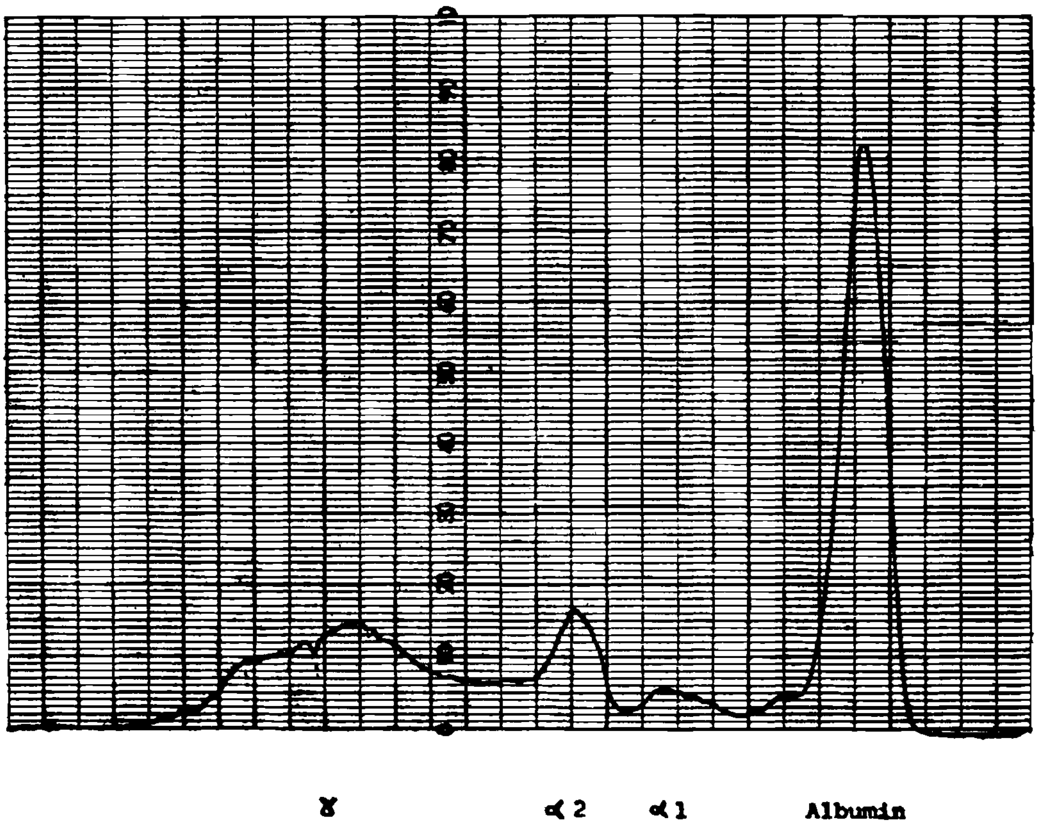

FIGURE 2

Electrophoretic separation of serum proteins of a control juvenile female chamaeleon.

Male chamaeleons were also treated with oestrogen and progesterone and with a combination of the two hormones. The results obtained cannot be directly compared with those obtained for females. In those cases where oestrogen and oestrogen plus progesterone were injected there appears to be a relative increase in the $\gamma$ globulins when these are compared with control animals. Due to insufficient sample numbers in the case of the males, a statistical analysis was not possible and no definite conclusions can be reached. It is, however, interesting to note that the electrophoretic separation of male serum proteins showed a $\beta$ peak in the majority of cases. This peak is mostly absent in females.

It is possible that oestrogen and oestrogen plus progesterone bring about an increase in the total proteins of the male chamaeleon. This assumption is backed by a significant increase in the liver weights relative to body weight of males treated with oestrogen plus progesterone. The mean relative liver weights increased significantly ($P < 0,05$) from 27,34 mg/g of body weight to 33,52 mg/g of body weight in animals receiving oestrogen plus progesterone.

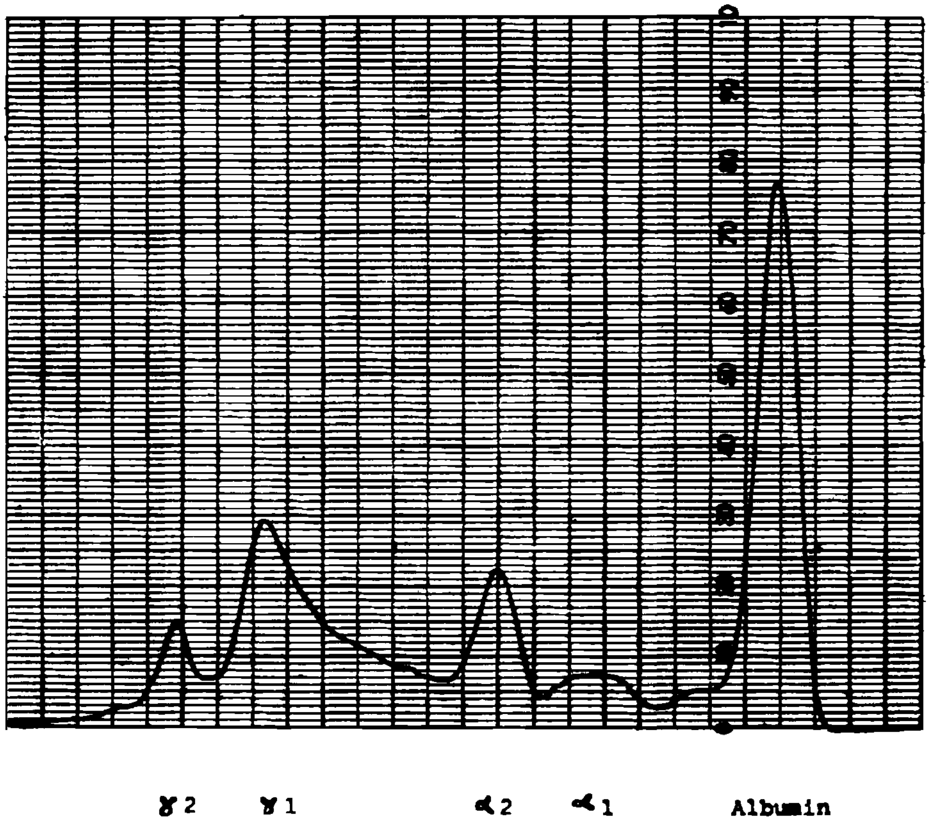

FIGURE 3

Electrophoretic separation of serum proteins of a juvenile female chamaeleon treated with oestrogen.

The electrophoretic separation of plasma proteins of adult female chamaeleons during various stages in the reproductive cycle, show that albumin, $\alpha$ 1, $\alpha$ 2, $\gamma$ 1 and $\gamma$ 2 are always present in the adult. The $\beta$ fraction is always absent in females.

Electrophoresis was carried out on the plasma of pregnant females, non-gravid females and non-gravid yolking females. A statistical analysis showed that the reproductive state of the chamaeleon does not affect the $\gamma$ plasma proteins significantly (Table 3). There is, however a slight but non-significant decline in the relative amounts of the $\gamma$ globulins in pregnant and yolking chamaeleons. This is probably due to transportation of $\gamma$ globulins from the blood to the follicles. This decline in the $\gamma$ globulins is compensated for by a significant increase ($P < 0,05$) in the $\alpha$ 1 globulins during the yolking stage and a significant increase in both $\alpha$ 1 and $\alpha$ 2 ($P < 0,05$) during pregnancy.

It appears then as if the livers of juvenile female chamaeleons do not produce the $\gamma$ globulin fractions which are present in the yolk. When the animals reach sexual maturity, oestrogens are

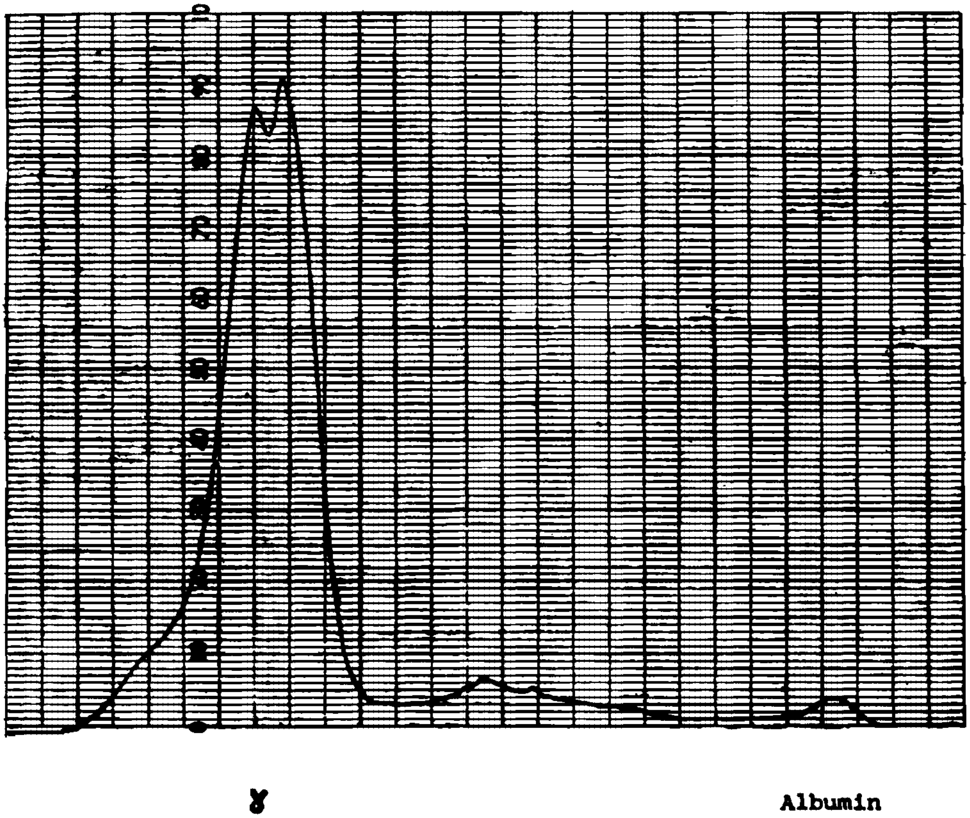

FIGURE 4

Electrophoretic separation of yolk present in the follicles of an adult chamaeleon.

produced during the follicular phase and stimulate the liver to produce the $\gamma$ 1 fraction, probably phosvitin. This could also occur via the pituitary as suggested by Gerstle & Callard (1972). This ability to produce the $\gamma$ globulins present in yolk is probably maintained for the rest of adult life, or conversely after reaching maturity there is a constant source of oestrogens to stimulate the production of these proteins.

TABLE 3

RESULTS OF ELECTROPHORESIS OF PLASMA OF NON-GRAVID, NON-GRAVID YOLKING AND PREGNANT CHAMAELEONS

| *Reproductive stage* | *Albumin %* | *$\alpha$1 (%)* | *$\alpha$2 (%)* | *$\gamma$1 (%)* | *$\gamma$2 (%)* |
|---|---|---|---|---|---|
| Non-gravid stage | 55,33 ± 9,07 | 6,27 ± 0,74 | 13,56 ± 1,53 | 14,55 ± 5,78 | 10,28 ± 4,07 |
| Yolking stage | 56,20 ± 4,73 | 8,26 ± 1,26 | 13,57 ± 2,93 | 12,81 ± 3,11 | 9,16 ± 2,36 |
| Gravid stage | 55,62 ± 2,59 | 8,46 ± 1,37 | 16,16 ± 1,90 | 12,09 ± 1,05 | 7,67 ± 3,43 |

### *The effect of progesterone on yolked follicles*

It is known that progestins inhibit ovulation in reptiles (Callard *et al.* 1972) and that they can cause yolk regression. Progesterone-induced yolk regression has been demonstrated in birds by Neher & Fraps (1950).

TABLE 4

EFFECT OF 0,05 MG PROGESTERONE/DAY ON FOLLICULAR DIAMETER

| *Number of follicles in ovaries before progesterone treatment* | *Number of follicles regressed after progesterone treatment* | *Number of follicles in which ovulation had occurred* | *Average follicular diameter before progesterone treatment (mm)* | *Average follicular diameter of regressed follicles after progesterone treatment (mm)* |
|---|---|---|---|---|
| 16 | 14 | 2 | 5,1 | 3,0 |
| 14 | none | 14 | 6,5 | — |
| 15 | 13 | 2 | 4,9 | 3,9 |
| 12 | none | 12 | 6,0 | — |
| 14 | 3 | 11 | 7,0 | 3,0 |
| 11 | 11 | none | 6,5 | 5,4 |
| 13 | 2 | 11 | 7,5 | 3,1 |

The daily injection of 0,05 mg progesterone during the present study brought about a variety of results. In some cases there was a regression of yolk in all the yolked follicles. However, if the follicles had a diameter of 6 mm or more at the time of laporotomy, then ovulation usually took place regardless of whether the animals received progesterone or not. Sometimes, certain follicles were more affected than others and a combination of the above factors also occurred, e.g. certain follicles regressed while ovulation occurred in others in the same ovary (Table 4). Control animals which received a daily injection of ethyl alcohol exhibited ovulation in all follicles. The follicular diameter of the controls ranged from 5 mm to 6,5 mm at the times of laporotomy.

The data contained in Table 4 indicate that progesterone causes regression of yolk in *C. pumilus* and it would appear to be the hormone which prevents the onset of the next follicular phase during gestation.

## EFFECT OF OVARIECTOMY AND PROGESTERONE REPLACEMENT THERAPY UPON GESTATION

In reptiles the necessity of the corpus luteum for the maintenance of gestation is in dispute (Clausen 1940; Bragdon 1951; Panigel 1956). Callard *et al.* (1972) found that for *Sceloporus cyanogenys* the course of gestation was unaffected by gonadectomy but that parturition was delayed or prevented. Burrage (1973) found that ovariectomy in *Chamaeleo pumilus* caused resorption of young in the early stages of development but that it had no effect during later stages of gestation.

In the present study twelve gravid females were ovariectomized and sham operations performed on a further six. Six of the ovariectomized group received 0,025 mg progesterone every third day, while the second group of six received an equivalent volume of the solvent (ethyl alcohol).

In all those injected with progesterone development of the embryos was unimpaired. Macroscopic examination of the oviduct showed that the blood supply to the oviduct had increased greatly. One of the females that received progesterone aborted one embryo shortly after ovariectomy, but this was probably due to post-operative shock as the development of the remaining embryos was normal. At autopsy all the embryos were alive, while the shape of the oviduct had been maintained and each embryo was located in a separate egg-chamber.

Sham-operated chamaeleons did not abort and development was normal, as in the group receiving progesterone. The results obtained for the ovariectomised group, not receiving progesterone, are given in Table 5. These results show that a large number of embryos in this group had died, some were in the process of being resorbed and in the case of one individual five embryos were aborted. Moreover, it was found that the uniform shape of the oviducal egg-chambers had deteriorated, frequently more than one embryo occupied a single egg-chamber and the blood supply to the oviducts had decreased.

The death of the embryos and the resulting large quantity of dead material in the oviducts, placed great stress on the mother, and if she was not able to abort these embryos or resorb some of them, she died within two weeks.

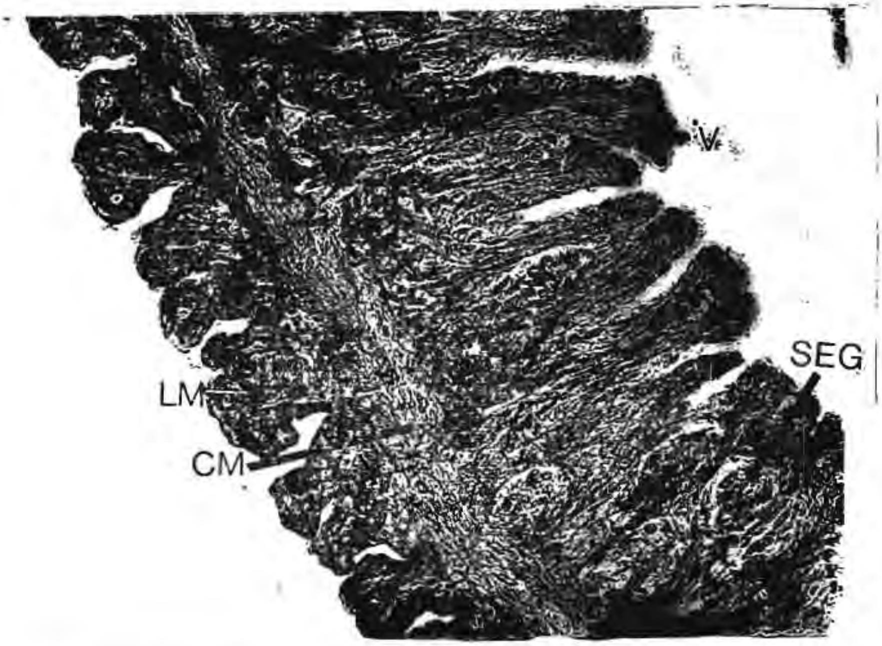

FIGURE 5
Cross section through the uterus showing longitudinal muscle layer (LM), circular muscle layer (CM), subepithelial glands (SEG) and villi (V).

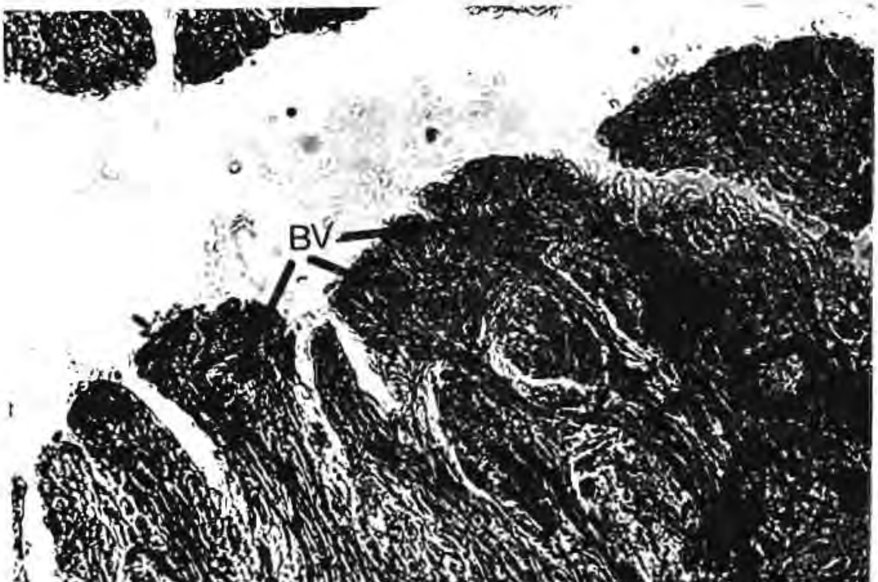

FIGURE 6
Cross section through uterus showing a villus with blood vessel (BV).

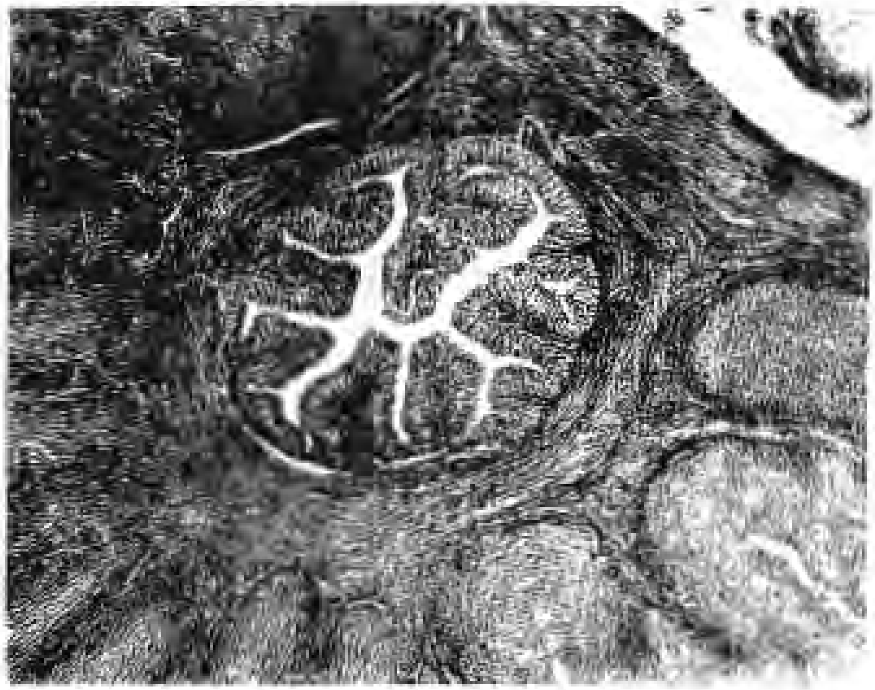

FIGURE 7

Cross-section through posterior vaginal area showing thick circular muscle layer (CM) and folded epithelium.

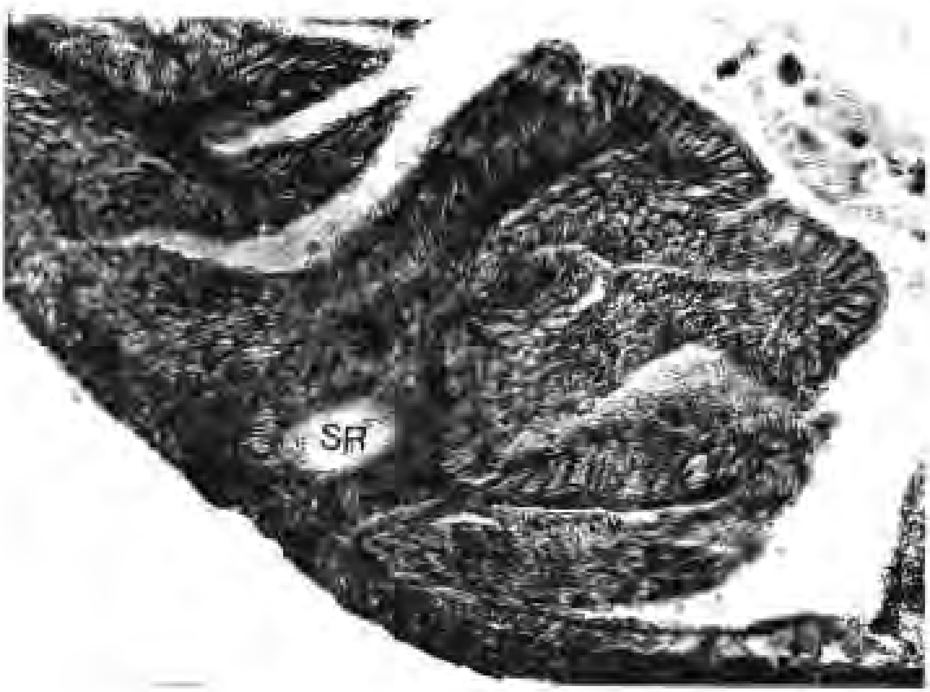

FIGURE 8

Cross section through mid-vaginal region showing a seminal receptacle (SR).

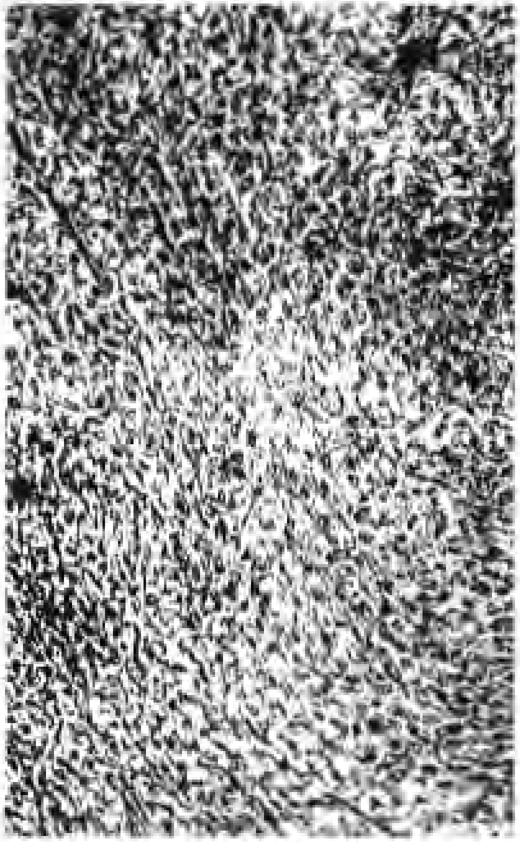

FIGURE 9
Cross section through a corpus luteum of a female in early gestation showing random orientation of luteal cells (× 250).

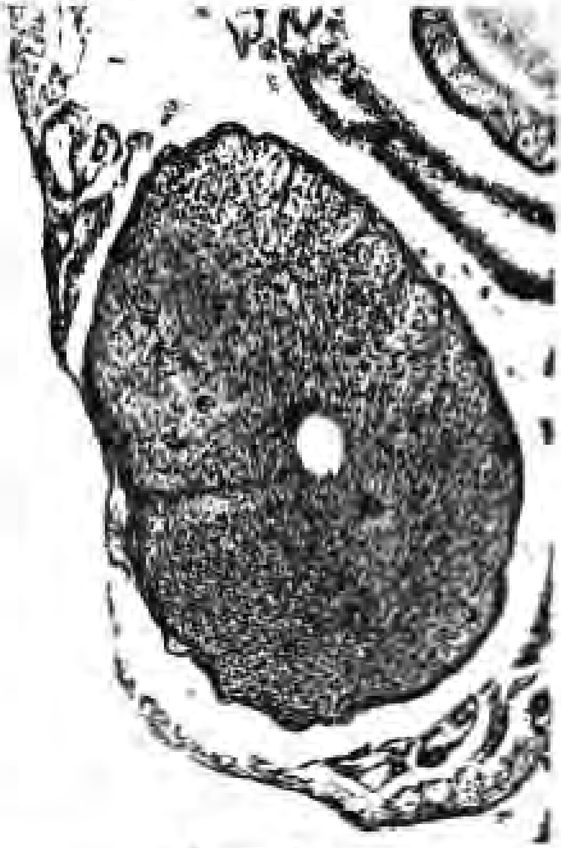

FIGURE 10
Cross section through the corpus luteum of a female in mid-gestation, showing a central cavity (× 100).

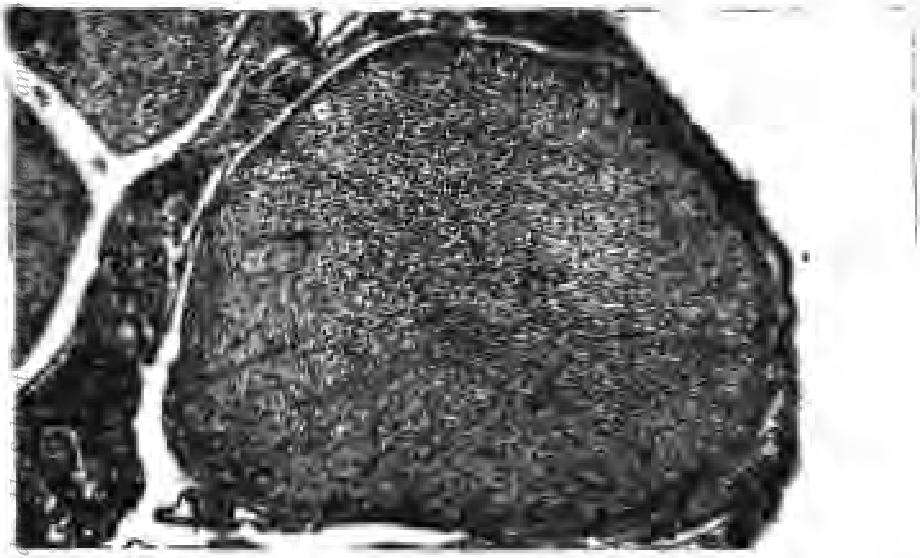

FIGURE 11
Cross section through corpus luteum of a female in late gestation showing compact luteal cells (× 200).

FIGURE 12
Cross section through a corpus luteum of a female one hour after parturition showing central degeneration of the lutein cells (× 100).

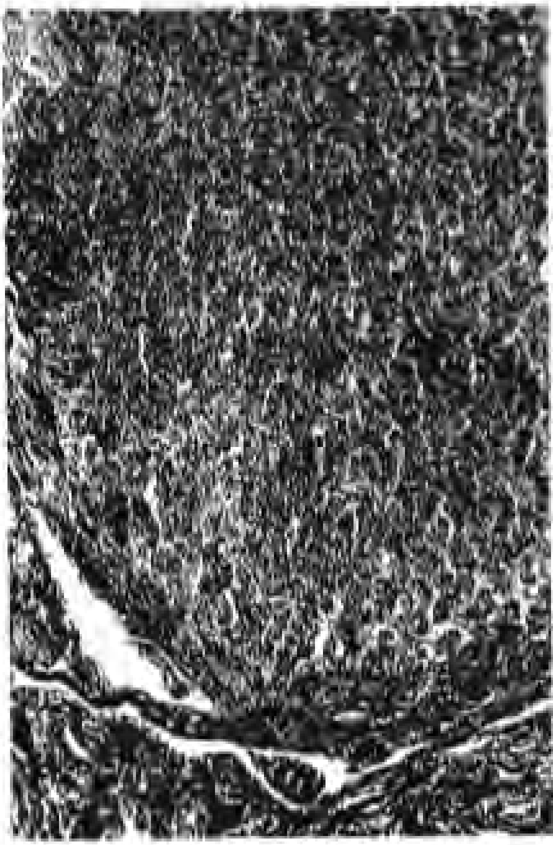

FIGURE 13
Tangential section through corpus luteum of a female one hour after parturition showing large intercellular spaces (× 250).

FIGURE 14
Cross section through a corpus albicans of a female one month after parturition showing isolated lutein cells cells surrounded by connective tissue (× 250).

It is possible that the death of the embryos was as a result of anoxia, due to reduction in the vascularity of the oviduct. Progesterone apparently prevents this hyporemia from taking place, and is thus an important factor during gestation in *C. pumilus*. Foetal death cannot be attributed to the exhaustion of the yolk supply as was found for *Sceloporus cyanogenys* (Callard *et al.* 1972) as all embryos still had sufficient yolk at the time of autopsy.

Also of interest is the fact that all the embryos in the ovariectomized group receiving progesterone remained in separate egg-chambers, while this was not the case in those not receiving the hormone. This indicates that progesterone is essential during pregnancy to maintain the integrity of the oviduct. It would appear then that the corpus luteum is essential for the maintenance of normal gestation in *C. pumilus* during early and mid-pregnancy.

TABLE 5

EFFECT OF OVARIECTOMY ON GESTATION IN *C. pumilus*

| *Number of embryos present before ovariectomy* | *Number of embryos being resorbed* | *Number of embryos aborted* | *Number of embryos dead* |
|---|---|---|---|
| 11 | 2 | — | 2 |
| 14 | 1 | — | 1 |
| 14 | — | — | 14 |
| 16 | — | — | 16 |
| 10 | — | 5 | 2 |
| 11 | 2 | — | 5 |

## SUMMARY AND CONCLUSIONS

The histological appearance of the oviduct was studied and sperm storage organs were found to be present in the vaginal region. The uterine section of the oviduct was found to be more vascular than the vaginal or infundibular regions. The blood vessels in the uterus are closely aligned with embryonic yolk-sac vessels and it was tentatively established that the transfer of nutrients may take place between the mother and the embryo.

A quantitative analysis of progesterone in the corpus luteum and circulating blood of *C. pumilus* by means of a radio-immuno assay showed that hormonal levels in this species are similar to those found in mammals and therefore probably have as important a function in *C. pumilus* as is the case in mammals.

The effect of progesterone and oestrogen on serum protein fractions and on oviducal histology was studied and it was found that oestrogens play an important part in the production of the $\gamma$ globulins present in yolk, with progesterone enhancing this effect. Oestrogens cause proliferation of the oviducal mucosa and work synergistically with progesterone to achieve this.

A study of the effect of progesterone on yolked follicles suggests that progesterone prevents the onset of the next follicular phase, as it causes yolk regression. The effect of ovariectomy and progesterone replacement therapy was also investigated and it was found that the corpus luteum and progesterone are essential for the maintenance of gestation in *C. pumilus*.

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* Not seen in the original.