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Structure and function of the oviduct in gekkonid lizards

Jane E. Girling

A thesis submitted for the degree of
Doctor of Philosophy
of the University of Otago, Dunedin
New Zealand

June, 1998
ABSTRACT

Although the structure of the oviduct has been analysed in several reptilian species using light microscopy, little ultrastructural information is available, particularly following hormonal manipulation. Here, oviductal structure and ultrastructure from wild females, and females surgically and hormonally manipulated in the laboratory, was analysed using light (LM), scanning electron and transmission electron (TEM) microscopy in three species from the lizard family Gekkonidae: *Hoplodactylus maculatus* (viviparous), *Saltuarius wyberba* (oviparous, producing a soft, parchment-like eggshell) and *Hemidactylus turcicus* (oviparous, producing a hard, calcareous eggshell).

The oviduct of the viviparous gecko *H. maculatus* exhibited seasonal changes. Maximal epithelial cell height was measured during vitellogenesis. The uterus exhibited the most obvious changes, due to the presence of a large, yolky egg following ovulation. Ultrastructural analysis identified features, such as bleb cells, apical protrusions and secretory granules, which were not visible at the LM level.

The uterus also exhibited the most distinct differences in a comparison of oviductal structure among vitellogenic females from the three gekkonid species. Viviparous *H. maculatus* had few uterine mucosal glands, whereas the oviparous species *S. wyberba* and *H. turcicus* had numerous glands (which secrete the eggshell membrane). The difference in gland density between the viviparous and oviparous species is consistent with other groups of reptiles in which viviparity has independently evolved. The number of secretory granules, and the staining properties of gland cells, differed between *S. wyberba* and *H. turcicus*. The differences may relate to the different types of eggshells produced by these species. Other differences among species, which could not be directly related to parity mode or eggshell type, may be related to the time period until ovulation for the individuals examined.

The literature shows that estradiol, secreted by the ovary during vitellogenesis, causes seasonal oviductal development in reptilian species; that ovariectomy, which removes the natural source of estradiol, causes oviductal regression; and that administration of estradiol to ovariectomised females causes oviductal development. The above patterns were confirmed in this study for *H. turcicus*. This is the first study to analyse the effects of exogenous estradiol on reptilian
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oviductal structure using TEM and to observe changes during the period of estradiol treatment. Changes observed included differentiation of the epithelium into ciliated and non-ciliated cells and increased numbers of secretory granules in non-ciliated cells.

Contrary to expectation, oviducts in ovariectomised *H. maculatus* were fully differentiated and secretory, resembling those of naturally vitellogenic females. No differences in oviduct structure at the LM and TEM level were observed among ovariectomised *H. maculatus* treated with vehicle solution only, estradiol, or estradiol followed by progesterone. Plasma estradiol concentrations were similar among groups. These results question the traditional view of estradiol-mediated oviductal development and suggest an extra-ovarian source of estradiol or that other hormones may maintain oviductal hypertrophy following ovariectomy in *H. maculatus*.

Immunocytochemistry was explored (ultimately unsuccessful) to try and determine the distribution of insulin-like growth factor-I (believed to facilitate the actions of estradiol) in the gekkonid oviduct.

Current literature and the findings of this study are incorporated into a review of oviductal structure and function in reptiles. Overall, this thesis provides comprehensive ultrastructural information including the identification of features not visible at the LM level and changes following hormonal manipulation.
ACKNOWLEDGMENTS

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When I first began my PhD, I planned to focus my research on the evolution of viviparity, a reproductive mode where the embryo is retained within the reproductive tract of the female until development is complete and birth can occur (Guillette, 1993). I wanted to determine the physiological and anatomical mechanisms by which viviparity evolved from oviparity (egg-laying). However, I had much to learn!

Within the four living orders of reptiles (Crocodilia, Sphenodontia, Squamata, Testudines), viviparous species are only found in the squamates. Within the squamates (lizards and snakes), viviparity is believed to have independently evolved from oviparity over 100 times (Blackburn, 1982; Blackburn, 1985). The ecological factors favouring the evolution of viviparity have long been a topic of debate (Tinkle et al., 1970; Blackburn, 1982; Shine, 1985; Shine, 1989), as have the possible physiological and anatomical changes required (G. Packard et al., 1977; I. Callard et al., 1992; Guillette, 1993). G. Packard et al. (1977) hypothesised that the retention of eggs within the oviduct for longer and longer time periods constituted an initial step in the evolution of viviparity. As a consequence, development of the embryo prior to oviposition is extended. G. Packard et al. (1977) further suggested that development within the oviduct was constrained by the need for gas exchange, hence the need for a placenta. The development of a placenta, which is defined as the intimate apposition or fusion of maternal with parental tissues for physiological exchange (Mossman, 1937), requires a reduction in eggshell thickness and the amount of calcification to allow close association of the extraembryonic membranes with the oviductal tissues. Reduced eggshell thickness is believed to be a result of the limited number of shell glands found in the uterus of viviparous species in comparison to oviparous species (for example: Guillette and Jones, 1985b). The question remains, however, as to how the shell glands are lost? Although we know that uterine shell glands develop during vitellogenesis in response to circulating estrogens (Christainsen, 1973; H. Fox, 1977), that is all we know. Guillette (1993) hypothesised that a reduction in the number of shell glands could result from a reduction in circulating estrogens, or a reduction in, or blocking of, estrogen receptors. Another unknown is how calcium secretion is controlled in the oviduct of oviparous species and what prevents calcium secretion in viviparous species.
Thus, the physiological and anatomical changes associated with the evolution of viviparity revolve around the oviduct and its functions. The oviduct, which is the adult organ derived from the embryonic Müllerian duct (Wake, 1985), provides passage for the egg after ovulation until oviposition or parturition. In oviparous species, it is responsible for secreting both the organic and inorganic (calcareaous) components of the eggshell. In viviparous species, the oviduct forms placental interactions with extraembryonic membranes. Although the processes by which the multiple evolutions of viviparity occurred in squamate reptiles can only ever be speculation, our hypotheses are limited by our restricted knowledge concerning the mechanisms of action of the oviduct. Thus, the aim of my research changed to increasing the knowledge available concerning the structure and function of the oviduct.

The reptilian oviduct is a multi-functional tissue. As well as playing a role in shell production and placental interactions, the oviduct is the site of sperm storage and fertilisation, it may be involved in albumen production, and it is involved in oviposition or parturition when hormonal and neural actions trigger oviductal contraction. The majority of reptiles have two oviducts, one lying on either side of the body and both entering a common urogenital opening (H. Fox, 1977; Fig 1.1b). The oviduct is divided into several different regions (starting anteriorly): infundibulum, uterine tube, isthmus, uterus and vagina (refer Fig. 3.1 for diagram). There is, however, some variation as to which regions are recognised and how authors define them. In general though, the infundibulum receives the ovulated egg which then moves down to the uterine tube where the albumen layer, if it is present, is secreted. The egg then moves via the isthmus into the uterus where it receives the eggshell components or, in the case of a viviparous species, placental interactions occur. During oviposition/parturition, the egg passes through the vagina and out via the common urogenital opening. Although several studies have analysed the gross morphology and structure of the oviduct using light microscopy (for example: H. Fox, 1977; Guillette and Jones, 1985b; Uribe et al., 1988; Palmer et al., 1993), there is only minimal ultrastructural information available, particularly ultrastructural changes over a reproductive cycle or in response to hormone manipulation.

Research undertaken in this thesis was carried out with species from the lizard family Gekkonidae. As currently classified, the family Gekkonidae consists of four subfamilies: Eublepharinae, Sphaerodactylinae, Gekkoninae and Diplodactylinae. The Diplodactylinae are further subdivided into two tribes,
Figure 1.1. New Zealand's common gecko, *Hoplodactylus maculatus* (H. n. sp. "Otago"). A: Pregnant female. B: Abdominal cavity of a pregnant female showing the two oviducts. The infundibulum, uterine tube, and isthmus are obscured from view. Both uteri (u) are visible and each contains a large, yolky egg. The vaginas (v) are also visible and lead to the common urogenital sinus (s). The gut (g) has been moved to one side to show an ovary (o) with a creamy coloured corpus luteum (arrow). Note black colour of oviductal tissues due to melanin.
Carphodactylini and Diplodactylini (Kluge, 1967). The tribe Carphodactylini includes the two New Zealand genera, Naultinus and Hoplodactylus. Within the Gekkonidae, viviparity is restricted to only three genera, all of which belong to the tribe Carphodactylini. This includes all of the New Zealand species belonging to the genera Naultinus and Hoplodactylus, as well as one species from the genus Rhacodactylus which is found in New Caledonia (Bauer, 1990). All other Carphodactylini, and all other geckos found worldwide, are oviparous. Another distinguishing factor between subfamilies is the type of eggshell produced. All members of the Diplodactylinae and Eublepharinae produce soft, parchment-like eggshells (Bustard, 1968), whereas members of the subfamilies Gekkoninae and Sphaerodactylinae produce hard, calcareous eggshells. This means the Gekkonidae are an excellent family in which to analyse oviductal structure and function as they exhibit viviparous and oviparous species, and additionally, the oviparous species produce various eggshell types. Although differences in oviductal structure between oviparous and viviparous species have been examined in other families of lizards, there has been no previous comparison for gekkonid species. The family Gekkonidae are also an appropriate choice from a New Zealand point of view. As yet, there is little detailed information on reproductive physiology available for New Zealand lizard species.

Over the course of my PhD research I have used three different gecko species for analysis: Hoplodactylus maculatus (Fig. 1.1a), Saltuarius wyberba and Hemidactylus turcicus. The first of the species used (Hoplodactylus maculatus, Carphodactylini, Diplodactylinae) is viviparous and belongs to a species complex. The females, which were collected from a population in central Otago, are part of a species referred to as H. n. sp. "Eastern Otago" by Daugherty et al. (1994), but as H. n. sp. "Otago" (includes both H. n. sp. "Eastern Otago" and H. n. sp. "Western Otago") by Hitchmough (1997). Species descriptions from the complex are yet to be published, therefore the population of geckos analysed here will still be referred to as H. maculatus.

The population of H. maculatus studied in this thesis exhibits an annual reproductive cycle with ovulation occurring in spring (September/October). This is followed by a gestation period of approximately 4 mo with parturition occurring in late summer (January/February). Yolking of the ovarian follicles for the next reproductive cycle begins just prior to parturition. This is a similar reproductive cycle to several other populations from the H. maculatus species complex (MacAvoy, 1976; Whitaker, 1982; Robinson, 1985), but differs from a
population approximately 11 km from the collection site where the females exhibit a biennial reproductive cycle with a gestation period of approximately 14 mo (Cree and Guillette, 1995). MacAvoy (1976) provided a brief overview of oviductal structure (using light microscopy only) in a population of *H. maculatus* considered to be the same species as that studied in this thesis (*H. n. sp. "Otago"). Additionally, an earlier study by Boyd (1942) used light microscopy to compare oviductal structure between non-pregnant and pregnant females, as well as uterine changes during placentation, in a different species from the *H. maculatus* species complex collected near Wellington (Hitchmough, 1997).

*Saltuarius wyberba* (Carphodactylini, Diplodactylinae) is a newly named species (Couper *et al.*, 1997) and to my knowledge, no reproductive information is available concerning this species. Females in the later stages of vitellogenesis were collected in October (spring) from Girroween National Park, Queensland, Australia. Species from the genus *Saltuarius* produce two, soft-shelled eggshells (Couper *et al.*, 1993).

The third species analysed, *Hemidactylus turcicus* (Gekkoninae), is also oviparous, but produces hard, calcareous eggshells (M. Packard *et al.*, 1982; M. Packard and Hirsch, 1986, 1989). The animals used in this study were collected from around Gainesville, Florida. Females in late vitellogenesis were found in May (Spring). In a population of *H. turcicus* studied in southern Louisiana, the reproductive season extended from April to August (Rose and Barbour, 1968). Two or three clutches were produced per season; about two months were required per clutch.

**THESIS AIMS**

The overall aim of this thesis is to examine aspects of oviductal structure and ultrastructure, and the response of the oviduct to steroid hormone treatment, in several members of the Gekkonidae using light, scanning electron and transmission electron microscopy. The particular objectives are to:

- describe the seasonal oviductal cycle in a viviparous gecko species (*Hoplodactylus maculatus* [H. n. sp. "Otago", Hitchmough, 1997])
- compare oviductal structure and ultrastructure among three gecko species exhibiting different parity modes (*H. maculatus*, viviparous; *Saltuarius*...
wyberba, oviparous and producing a soft, parchment-like eggshell; 
*Hemidactylus turcicus*, oviparous and producing a hard, calcareous eggshell)

- determine the structural and ultrastructural changes occurring in uterine tissues of an oviparous species (*H. turcicus*) after treatment of ovariectomised females with estradiol
- examine the effects of estradiol and progesterone treatment following ovariectomy on oviductal structure and ultrastructure in the viviparous gecko, *H. maculatus*
- determine the distribution of insulin-like growth factor-I in oviductal tissues of *H. maculatus* and *H. turcicus*
- review current literature concerning the structure and functions of the oviduct in reptiles, including the research findings of this thesis.

**STRUCTURE OF THE THESIS**

Chapters 2-7 of this thesis are self-contained, but inter-related, pieces of work. This means some repetition is unavoidable. Chapter 8 provides the overall conclusions of the research undertaken in this thesis. The material contained in chapters 2-5 has been published or is to be published, as indicated below, with Dr Alison Cree and Professor Louis Guillette, Jr. as co-authors. Chapter 7 will also be submitted for publication as a sole-authored work. The chapters have undergone minor editing to suit the thesis format. This includes cross-referencing by chapter number rather than publication details where work elsewhere in the thesis is referred to. A single acknowledgments and references section has been compiled.

Ethics approval was gained from the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals (68-94, 50-95, 8-97).

**Chapter Two**

Oviductal structure in a viviparous New Zealand gecko, *Hoplodactylus maculatus*.


*Journal of Morphology*, 234: 51-68.

**Chapter Three**

Oviductal structure in three species of gekkonid lizard exhibiting different parity modes.


*Reproduction, Fertility and Development*, accepted subject to revision.
Chapter Four
Ultrastructure of the uterus in an ovariectomised gecko (Hemidactylus turcicus) after administration of exogenous estradiol.
Girling, J.E.; Guillette, L.J., Jr. and Cree, A.
Submitted to the Journal of Experimental Zoology.

Chapter Five
Unexpected results in a study analysing the effects of progesterone on oviductal structure in estrogen-primed New Zealand common geckos (Hoplodactylus maculatus).
To be submitted to General and Comparative Endocrinology.

Chapter Six
Detection of insulin-like growth factor-I in the gekkonid oviduct using immunocytochemistry.
Not for publication.

Chapter Seven
Structure and function of the reptilian oviduct.
Girling, J.E.
To be submitted for publication (journal not yet decided).

Chapter Eight
General Conclusions
Not for publication
Chapter Two

Oviductal structure in a viviparous New Zealand gecko, *Hoplodactylus maculatus.*
ABSTRACT

Oviductal structure was described in New Zealand’s common gecko, *Hoplodactylus maculatus*, over four reproductive stages (early/mid vitellogenesis, late vitellogenesis, early pregnancy, late pregnancy), using light, scanning electron and transmission electron microscopy. Five regions of the oviduct were recognised: infundibulum, uterine tube, isthmus, uterus and vagina. Up to three cell types made up the luminal epithelium of the oviduct: ciliated, non-ciliated, and bleb cells. The function of bleb cells (seen in the infundibulum only) is unknown, but observation of these cells using transmission electron microscopy suggests that they are involved in secretory activity. Mucosal glands in the uterine tube possessed large numbers of secretory granules of varying electron densities. Additionally, these glands appeared to function as sperm storage tubules. Numerous sperm were seen in the glands during late vitellogenesis and early pregnancy. Very few uterine mucosal (shell) glands were seen during vitellogenesis, which is consistent with the observation that only a fine shell membrane covered the egg during early pregnancy. By late pregnancy, extra-embryonic membranes lay adjacent to the uterus allowing the formation of the omphalo- and chorioallantoic placentas. Maximum cell height in the luminal epithelium was seen during vitellogenesis. The maximum percentage of ciliated cells making up the epithelial layer was seen during pregnancy. The low number of uterine mucosal glands seen in *H. maculatus* is a feature typical of other viviparous reptiles described, despite independent evolutions of viviparity. Although oviductal structure has been described in the literature for various reptiles, several ultrastructural features seen in this study highlight the lack of detailed understanding of this tissue.
INTRODUCTION

The structure of the reptilian oviduct has been studied in a variety of species (for example, lizards: Uribe et al., 1988; Guillette et al., 1989; snakes: Mead et al., 1981; Perkins and Palmer, 1996; turtles: Palmer and Guillette, 1988; Sarker et al., 1995; crocodilians: Palmer and Guillette, 1992), although few studies have analysed oviductal ultrastructure using transmission electron microscopy (Aitken and Solomon, 1976; Palmer and Guillette, 1992; Gist and Fischer, 1993). In reptiles, the term 'oviduct' refers to structures derived from the embryonic Müllerian duct system (Wake, 1985). Along the length of the oviduct, several regions have been distinguished (H. Fox, 1977). At the anterior end, the infundibulum receives the ovulated egg via an ostial opening. The infundibulum leads into the uterine tube, and then through a tubal-uterine junction (isthmus) into the uterus. Most posterior is the vagina, which leads into a common urogenital sinus (cloaca, H. Fox, 1977). These terms should not be confused with those used in the mammalian literature where the term oviduct is synonymous with only the upper portion of the tract, i.e. the Fallopian tube, and excludes the uterus and vagina. All five oviductal regions are not recognised in every reptilian species examined and/or further divisions of the reptilian oviduct may be included. For instance, Shanthakumari et al. (1990) considered the uterine tube to be the posterior part of the infundibulum in the agamid lizard, Calotes versicolor. In alligators, the uterus was divided into two functionally distinct regions, the anterior and posterior uterus, which produced the fibrous membrane and calcareous layer of the eggshell, respectively (Palmer and Guillette, 1992). In contrast, the lizard Sceloporus woodi had a single uterine region, which was responsible for the secretion of both shell fibres and calcium (Palmer et al., 1993).

The reptilian oviduct can be divided into three tissue layers, which vary in thickness and function in different regions (O. Cuellar, 1966; H. Fox, 1977; Wake, 1985). The inner layer or mucosa is subdivided into an epithelial layer of ciliated and non-ciliated cells that line the lumen of the oviduct, and an underlying lamina propria, which contains connective tissue, blood vessels and any glands present. The middle layer of the oviduct is a region of smooth muscle (muscularis) that has an inner circular and/or outer longitudinal muscle layer. The outermost layer is termed the serosa and is continuous with the peritoneum.

All of New Zealand’s gecko species (belonging to two genera, Hoplodactylus and Naultinus) are viviparous. All other geckos known worldwide are oviparous,
Oviductal structure in a viviparous gecko

except one species of *Rhacodactylus* found in New Caledonia (Bauer, 1990). Blackburn (1982) and Shine (1985) suggested two independent evolutions of viviparity in the Gekkonidae, one in the ancestors of the New Zealand genera, and another in New Caledonia. In oviparous species, the oviduct is responsible for secretion of the eggshell and in viviparous species, it is associated with the extra-embryonic membranes forming the placenta. Thus, differences seen in oviductal structure between oviparous and viviparous species provide important information about the evolution of viviparity (see Guillette, 1993).

Very little is known about oviductal structure in the lizard family Gekkonidae. The oviduct of the oviparous gecko *Tarentola mauritanica* has been studied over a reproductive cycle using light microscopy (Picariello et al., 1989). Seasonal changes in the oviduct were described, with a maximum oviduct-somatic index occurring in May-June (spring). A brief overview of oviductal structure (using light microscopy only) in a gecko from the *Hoplodactylus maculatus* species complex was provided by MacAvoy (1976). An earlier study by Boyd (1942) used light microscopy to compare oviductal structure between non-pregnant and pregnant females, as well as uterine changes during placentation, in another species from the *H. maculatus* complex. The population of *H. maculatus* studied in this paper is thought to be same as that studied by MacAvoy (1976), but different from that studied by Boyd (1942; Daugherty *et al.*, 1994; Hitchmough, 1997). As species descriptions from the species complex are not yet published, the population studied here is still referred to as *H. maculatus*.

In this study I observed the structure of the oviduct in the viviparous gecko *Hoplodactylus maculatus* at four different reproductive stages (early/mid vitellogenesis, late vitellogenesis, early pregnancy and late pregnancy) using light, scanning electron and transmission electron microscopy. My aim was to provide a comprehensive description of changes in oviductal structure in a viviparous gecko over a reproductive season.

METHODS AND MATERIALS

Animal Collection

Geckos were collected near the Hyde turn-off from the Macraes Flat-Middlemarch Road in Central Otago, New Zealand. They were captured from under loose rock
slabs on schist outcrops at approximately 400 m above sea level. My collections indicated that the population at this site has an annual reproductive cycle with ovulation occurring in September/October (spring), followed by a gestation period of approximately 4 months. The ovarian follicles for the next reproductive cycle begin yolking just prior to parturition in January/February (late summer). This is similar to the reproductive cycle described for populations of *Hoplodactylus maculatus* from Alexandra (MacAvoy, 1976) and Turakirae Head (Whitaker, 1982; Robinson, 1985). However, the reproductive cycle for my population is different from that of a nearby population (approximately 11 km away) living at a higher altitude (approximately 500-700 m a.s.l.), where a biennial ovarian cycle, with an approximately 14 month gestation period, has been described (Cree and Guillette, 1995; A. Cree, unpublished observation). Four reproductive conditions were collected: early/mid vitellogenesis (March, 1995; n=6), late vitellogenesis (September, 1995; n=5), early pregnancy (October, 1994; n=6) and late pregnancy (February, 1995; n=6). Reproductive condition was initially determined by gentle palpation of the abdomen (Cree and Guillette, 1995) and confirmed upon dissection. In vitellogenic females, the follicles were firm and spherical. Early/mid vitellogenic females (follicle diameter 6-10 mm) were collected prior to winter and late vitellogenic females (follicle diameter 7-11 mm) after winter. In early pregnant females, the ova were soft and oval in shape (maximum diameter 9-11 mm). By late pregnancy, the form of the embryo could be felt, and movements by the embryo were often seen (15-17 mm maximum diameter of oviductal egg). Mature females dissected ranged from 62-77 mm snout-vent length. Geckos were killed by decapitation (swift blow to the head followed by severance of the spinal column).

**Histology**

One oviduct from each female was preserved in Bouin's fixative. Where possible, the oviduct was processed *in situ* to retain its shape and proximity to other tissues. The tissues were washed and dehydrated in ethanol, (70%, three x 100%), cleared in xylene, embedded in paraffin, serially sectioned (6-8 μm) and mounted on glass slides. Three slides from every 10 were stained using a variety of procedures to visualise changes along the entire length of the oviduct. One of every 10 slides was stained with Lillie-Mayer Haematoxylin and Eosin (H&E, general histology) with an Alcian Blue 8GX (pH 2.5) counterstain (Humason, 1979). At this pH, Alcian Blue stains both carboxylated and sulfated acid mucosubstances, including mucopolysaccharides and mucoproteins. The second
of 10 slides was stained with Mallory's Trichrome (general histology and connective tissue), and the third with Periodic Acid-Schiff's Reagent (PAS, carbohydrate and carbohydrate-protein substances) with a Fast Green counterstain (Humason, 1979). Representative slides from each region were subjected to the saliva (diastase) test prior to PAS treatment (Humason, 1979). No differences between the slides treated with or without the saliva test were noted, therefore eliminating glycogen as the staining product. Some of the remaining slides were put aside for analysis of tissues for insulin-like growth factor-I using immunocytochemistry (chapter six).

Scanning and Transmission Electron Microscopy

The second oviduct was dissected out of the body cavity and cut with a sharp razor blade into the four regions visible with the naked eye: infundibulum, uterine tube, uterus and vagina (except females in early pregnancy [the first animals collected], in which the oviduct was cut into three regions: infundibulum/tube, uterus and vagina, based on information from Boyd (1942) and MacAvoy (1976)). In general, the isthmus (a very short region) was destroyed by cutting during preparation for electron microscopy and was only observed using light microscopy. Each tissue region was cut into small pieces (approximately 1 mm²) and separated for use in scanning and transmission electron microscopy. Tissues were initially preserved in 2.5% glutaraldehyde in phosphate buffer for 2.5 h, followed by post-fixation in 1% osmium tetroxide/1.5% potassium ferrocyanide in cacodylate buffer for 1 h. Tissues for scanning electron microscopy (SEM) were dehydrated down an ethanol series and critical-point-dried in liquid CO₂. The samples were then mounted and coated with gold palladium and viewed using a Cambridge S360 microscope at 20 kV. Tissues for transmission electron microscopy (TEM) were dehydrated down an ethanol series and infiltrated with agar resin. Semi-thin and ultra-thin sections were cut using an Ultracut E (Leica Instruments Pty Ltd, Australia). Samples were viewed on a Philips EM410 microscope.

Morphometrics and Statistics

Measurements of epithelial cell height, cilia length and the percentage of ciliated cells making up the epithelial layer were taken from paraffin sections mounted for light microscopy, using the Sigma Scan (version 3.9) measuring system and
digitizing tablet (Jandel Scientific, U.S.A.). For each variable, measurements were taken from three separate slides, if three slides were available for the tissue in question. From each of the slides, epithelial cell height and cilia length were measured in 30 randomly chosen cells. Each cell used had to show both apical and basal surfaces in the plane of section. To determine the percentage of ciliated cells making up the epithelial layer, 25 randomly chosen cells from each of three slides (if possible) were categorised as either ciliated or non-ciliated cells.

Using a 25-point grid (20 mm² squares) on scanning electron micrographs, the area taken up by ciliated cells in the epithelial layer was estimated by determining the cell type on each cross-point of the grid. Up to five grids (whenever possible) were counted per oviductal region per animal.

All morphometric analyses were tested using a general linear model (using SAS, Version 6.10, SAS Institute Inc., Cary, North Carolina), after log transformation to achieve homogeneity of variances if required. Fisher's protected LSD test was used as a post-hoc test if a significant difference was found. Animals nested within stage was the standard error term. All means are reported plus or minus one standard error.

RESULTS

General Description

During early/mid vitellogenesis, the oviduct was a fine, ribbon-like structure lying close to the posterior, dorsal body wall. It was connected to the peritoneum by the mesotubarial mesentery. The abdominal cavity of late vitellogenic females was dominated by up to two large ovarian follicles, one in each ovary. During pregnancy, the large, yolky eggs and embryos caused the uterine region of the two oviducts to dominate the body cavity (Fig. 1.1). The infundibulum and uterine tube curled back on themselves over the uterus and were greatly reduced in size when compared to the uterus. Melanin was present in the serosal layer of the oviduct (except in the vagina) giving it a black appearance to the naked eye.

In the population of *Hoplodactylus maculatus* studied here, five separate regions of the oviduct were distinguished using light microscopy: infundibulum, uterine tube, isthmus, uterus and vagina. In the luminal epithelium, up to three cell
types were present in each region: ciliated cells, microvillous non-ciliated cells, and bleb-like non-ciliated cells (bleb cells). Ciliated cells (in all regions examined) were characterised by a prominent nucleus. The nucleus was surrounded by a distinctive, dark nuclear membrane enclosing aggregations of chromatin (dark patches) and a nucleolus (this was not always visible, depending on the plane of section) (Fig. 2.1d). Mitochondria and free ribosomes were numerous. Each region and the nature of the luminal epithelium are described further below (summarised in Table 2.1).

**Infundibulum**

The infundibulum was a fine-walled region (Fig. 2.1a). It had a thin but distinct muscle layer, although the circular and longitudinal components of this were difficult to delineate. Very little connective tissue was present in the mucosa. The infundibulum opened to the body cavity via a funnel-shaped ostial opening. The luminal epithelium extended back over the lip of the ostium until it abruptly met the serosa. The epithelium at the lip of the ostium was predominantly ciliated, but had occasional non-ciliated cells. This trend was reversed posteriorly. In the anterior infundibulum the mucosa formed low folds, which gradually increased in height towards the uterine tube (Fig. 2.1a).

**Vitellogenesis**

During vitellogenesis the ciliated epithelial cells were cuboidal with nuclei situated basally. The ciliated epithelial cells became more columnar at the posterior end of the infundibulum (Fig. 2.1a). During vitellogenesis, this region was also characterised by large, non-ciliated cells, which sometimes partially obscured adjacent ciliated cells and that often formed apical protrusions into the lumen. The non-ciliated cells contained large cytoplasmic secretory granules of varying electron densities, which tended to be at, or near, the apical surface (Fig. 2.1d). The nucleus resembled that seen in ciliated cells. Mitochondria were seen aggregated in cell areas not dominated by the nucleus or secretory granules. The non-ciliated cells were covered with numerous microvilli and some had a single, long cilium which may indicate cells changing to/from ciliated cells (Fig. 2.1b).

Occasionally, small regions of bleb cells were observed (Fig. 2.1c). The blebs exhibited large, smooth apical surfaces which protruded into the lumen. These were easily identified with the SEM, but were more difficult to distinguish from
Table 2.1. Epithelial and mucosal features of the oviduct in *Hoplodactylus maculatus*: variations between vitellogenic and pregnant females.

<table>
<thead>
<tr>
<th>REGION</th>
<th>FEATURES</th>
<th>CHANGES</th>
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<tbody>
<tr>
<td>Infundibulum</td>
<td>Epithelium: ciliated, non-ciliated and occasional bleb cells. Non-ciliated cells: Alcian blue (acid mucosubstances) negative, slightly PAS (carbohydrate) positive.</td>
<td>VITELLOGENESIS: Non-ciliated cells: numerous microvilli and secretory granules. PREGNANCY: Non-ciliated cells: protrude into lumen, fewer microvilli.</td>
</tr>
<tr>
<td>Vagina</td>
<td>Epithelium: ciliated and non-ciliated cells (including some goblet cells). Non-ciliated cells: numerous secretory granules, PAS (carbohydrate) and Alcian Blue (acid mucosubstances) positive.</td>
<td>EARLY VITELLOGENESIS: Large numbers of sperm present in lumen. PREGNANCY: Occasional individual sperm seen in lumen</td>
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Figure 2.1. Infundibulum of *Hoplodactylus maculatus*. A: Cross section of infundibulum during early vitellogenesis showing the epithelium (e), lumen (l) and muscle layer (m). H&E with Alcian Blue. Scale bar = 100 μm. B: Epithelium during early vitellogenesis with both ciliated (c) and non-ciliated (n) cells. Note single cilium (arrow) and bleb-like secretory processes (p). SEM. Scale bar = 10 μm. C: Luminal epithelium during early vitellogenesis showing all three cell types including bleb (b) cells. SEM. Scale bar = 10 μm. D: Non-ciliated epithelial cells containing numerous secretory granules (s) during late vitellogenesis. TEM. Scale bar = 10 μm. E: Luminal epithelium during early pregnancy showing apical protrusions (a) "pinching" off into the lumen. SEM. Scale bar = 10 μm. F: Bleb cell and bleb-like secretory granules during early pregnancy. TEM. Scale bar = 5 μm. G: Enlargement of figure 2.1d. Scale bar = 10 μm.
Oviductal structure in a viviparous gecko
Oviductal structure in a viviparous gecko
large non-ciliated cells using the light microscope. TEM examination revealed a variety of apical protrusions, most of which had at least a few microvilli. The most likely candidates for bleb cells were those with a fine apical membrane which protruded into the lumen (Fig. 2.1f). These protrusions contained fine, non-granular material. The cytoplasm of the bleb cells contained normal cellular material, often with secretory granules. Other, smaller blebs could be seen protruding from epithelial cells, both ciliated and non-ciliated (Figs. 2.1b,c,f). The relative number of bleb cells present (based on tissue processed for SEM) was hard to determine as they were found on very few tissue pieces and were not seen in every animal, or at any particular reproductive stage. The presence of large non-ciliated and bleb cells gave the epithelium an irregular appearance (Fig. 2.1a). Neither non-ciliated nor bleb cells stained with Alcian blue (for acid mucosubstances), although they occasionally stained positively with the PAS procedure at the apical surface of the epithelium, suggesting the secretory granules contained some form of carbohydrate.

Pregnancy

Changes in the morphology of the epithelium of the infundibulum were seen during early and late pregnancy, in comparison to vitellogenesis. Bleb cells were again only rarely seen, and sometimes appeared depleted and shrunken. Ciliated cells often appeared pitted when viewed using the SEM (Fig. 2.1e), and in some cases a small secretory granule was present amongst the cilia of a single cell. On occasions, whole non-ciliated cells protruded into the lumen (nuclei included as seen using TEM), suggesting whole cells were budding off (Fig. 2.1e). Further evidence of this phenomenon was provided by the presence of nuclei and debris within the lumen. During pregnancy, non-ciliated cells had fewer microvilli present than during vitellogenesis. In some regions, groups of cells (usually non-ciliated) formed pear-shaped projections separated by a large ciliated cell.

Uterine Tube

The uterine tube was relatively thin walled. Layers of circular and longitudinal muscle were visible (Fig. 2.2a). The mucosa lay in longitudinal folds of uneven height; the epithelium lining these folds was supported by a underlying layer of connective tissue. The bases of these folds formed alveolar glands, the epithelium of which did not stain with either of the carbohydrate stains, PAS or
**Figure 2.2.** Uterine tube of *Hoplodactylus maculatus*. A: Cross section of uterine tube showing luminal epithelium during late vitellogenesis with glands (g), the lumen (l), and muscle layer (m). H&E with Alcian Blue. Scale bar = 50 μm. B: Cross section of posterior uterine tube during late vitellogenesis with glands acting as sperm storage tubules (t). H&E with Alcian Blue. Scale bar = 25 μm. C: Luminal epithelium during late vitellogenesis. Note non-ciliated cells with numerous microvilli (n), ciliated cells (c), and occasional sperm (arrow). SEM. Scale bar = 10 μm. D: Luminal epithelium during early pregnancy. SEM. Scale bar = 10 μm. E: Luminal epithelium during late pregnancy. Note numerous secretory granules (s). TEM. Scale bar = 1 μm.
Alcian blue (Fig. 2.2a). Gland cells contained large secretory granules of varying electron densities.

**Vitellogenesis**

The epithelial layer had both ciliated and non-ciliated cells. Epithelial cells in the uterine tube were narrow and columnar in appearance, with nuclei generally situated basally. Non-ciliated cells had few microvilli during early vitellogenesis. The few microvilli present occurred around the boundary of the apical surface. In contrast, non-ciliated cells were covered in microvilli during late vitellogenesis and the apical surfaces bulged out toward the lumen (Fig. 2.2c). The non-ciliated cells contained large numbers of secretory granules, which tended to be found throughout the cytoplasm (Fig. 2.2e). Non-ciliated cells stained positively with both the carbohydrate stains, Alcian blue and PAS. They also stained pale blue with Mallory's trichrome suggesting the presence of a carbohydrate-containing mucin. Figure 2.3 illustrates the colours associated with the different staining procedures.

Sperm were seen at the posterior end of the uterine tube, in bundles inside the alveolar glands (Fig. 2.2b) during late vitellogenesis. The number seen on the epithelial surface varied between animals.

**Pregnancy**

Few obvious changes were noted in the tubal epithelium during pregnancy. The epithelial cells were more cuboidal, with centrally situated nuclei. Non-ciliated cells had few microvilli (Fig. 2.2d). A few sperm remained in the alveolar glands during early pregnancy.

**Isthmus**

The isthmus was a short region separating the uterine tube from the uterus, and could not be recognised using the naked eye. During vitellogenesis the isthmus closely resembled the uterus. That is, the thickness of muscle and mucosal layers were similar and occasional glands were present. However, unlike in the uterus, epithelial cells did not stain with either of the carbohydrate stains, PAS or Alcian blue. The general appearance of the isthmus remained the same during pregnancy.
Figure 2.3 Paraffin sections of uterine tube from *Hoplodactylus maculatus* stained using three different procedures. **A:** Haematoxylin and Eosin (general histology) with an Alcian Blue counterstain (pH 2.5, carboxylated and sulfated acid mucosubstances). Notice the bright blue colour of secretory material within non-ciliated cells that has stained with Alcian Blue. **B:** Mallory’s Trichrome (general histology and connective tissue). Pale blue staining of secretory material within non-ciliated cells suggests the presence of mucin. **C:** Periodic Acid-Schiff’s Reagent (PAS, carbohydrate and carbohydrate-protein substances) with a Fast Green counterstain. Notice the dark pink colour of secretory material within non-ciliated cells that has stained with PAS. g: gland, l: lumen, m: muscle layers, n: non-ciliated cells, t: connective tissue. Scale bars = 50 μm.
Oviductal structure in a viviparous gecko
Uterus

Vitellogenesis

The uterus changed dramatically over a reproductive season. During vitellogenesis the uterus was thick-walled (Fig. 2.4a). The two muscle layers (inner circular and outer longitudinal) were distinct. The mucosa was thick and folded in a generally longitudinal direction. These folds were supported by a thick layer of connective tissue. Alveolar glands lined with non-ciliated cells occurred in the connective tissue of the mucosa (Figs. 2.4a, 2.5c,d), but were few in number and widely spaced. These glands contained secretory granules of varying electron densities (Fig. 2.5d), but did not stain with either of the carbohydrate stains, PAS or Alcian blue. The glands opened onto the luminal epithelium (Fig. 2.5c). The mucosa was well vascularised with small blood vessels under the epithelial layer. The epithelium was made up of columnar ciliated and non-ciliated cells (Fig. 2.5e). Nuclei were situated basally. The non-ciliated cells stained slightly with Alcian blue for acid mucosubstances, but intensely with PAS for carbohydrate. The stain was concentrated above the nuclei (i.e., only the apical half of the secretory cell stains). This corresponded with the large numbers of secretory granules seen in the apical regions of the cells under the TEM (Fig. 2.5f). Individual non-ciliated cells tended to have granules of a similar electron density, although adjacent cells could be quite different.

Sperm were sometimes seen in the lumen of the uterus during late vitellogenesis, presumably as they travelled to the uterine tube. The abundance of sperm varied among individuals.

Pregnancy

During early pregnancy the uterine region was highly stretched owing to the presence of a large yolky egg (Fig. 2.4b). The muscle layer was very thin, and no connective tissue was visible in the mucosa. No glands were seen. The epithelium was very thin, more so when covering a blood vessel in the underlying connective tissue. Ciliated cells were relatively sparse and the apical surface was concave in shape due, presumably, to pressure from the adjacent non-ciliated cells (Fig. 2.5a,b). Most of the ciliated cell's volume was filled with the nucleus. Non-ciliated cells no longer contained large secretory granules (Fig. 2.5b). They were packed with mitochondria and at the apical surface numerous
Figure 2.4. Uterus of *Hoplodactylus maculatus*. Cross section during A: Late vitellogenesis showing the lumen (l), the epithelial layer (e), glands (g), muscle layer (m) and connective tissue (t). PAS with Fast Green. B: Early pregnancy with uterus lying adjacent to extra-embryonic membranes surrounding yolk (y) supply, and C: Late pregnancy with uterus lying adjacent to extra-embryonic membranes (v). Arrow points to a blood vessel under the epithelial layer. B and C: H&E with Alcian Blue. Scale bars all 100 μm.
Oviductal structure in a viviparous gecko
Figure 2.5. Uterus of *Hoplodactylus maculatus*. A: Luminal epithelium during early pregnancy with ciliated (c) and non-ciliated (n) cells. SEM. Scale bar = 10 μm. 

B: Luminal epithelium during early pregnancy. TEM. Scale bar = 10 μm. 

C: Luminal epithelium during early vitellogenesis showing gland (g) opening. SEM. Scale bar = 5 μm. 

D: Luminal epithelium during early vitellogenesis with gland in underlying connective tissue. TEM. Scale bar = 10 μm. 

E: Luminal epithelium during late vitellogenesis. SEM. Scale bar = 10 μm. 

F: Luminal epithelium during late vitellogenesis showing numerous secretory granules (s). TEM. Scale bar = 10 μm.
Oviductal structure in a viviparous gecko
small membrane-bound vesicles were present. Microvilli on non-ciliated cells were short and bent close to the cell surface (Fig. 2.5a). The epithelium lay adjacent to a thin, fibrous shell membrane (seen using SEM).

The uterus remained very thin during late pregnancy (Fig. 2.4c). The muscle and connective tissue layers were thin, but distinctive. The epithelium could be extremely thin, especially when covering an underlying blood vessel in the mucosa. Large secretory granules of a uniformly low electron density were present, predominantly at the basal surface of the epithelial cells. Cells also contained some mitochondria and membrane-bound vesicles. The epithelium was now adjacent to extra-embryonic membranes forming the chorioallantoic and omphalo-placentas. Placental structure was analysed by Boyd (1942) in another member of this species complex and will not be discussed further here.

**Vagina**

In this region the mucosa was surrounded by a thick layer of circular muscle (Fig. 2.6a). The mucosa was thrown into longitudinal folds; these effectively reduced the volume of the lumen. The folds were supported by a distinctive lamina propria. The epithelial layer was predominantly ciliated (Fig. 2.6c). Epithelial cells were cuboidal to columnar with nuclei situated basally or medially. Non-ciliated cells contained secretory granules of varying electron densities (Fig. 2.6c), typically present at the apical surface. Goblet cells were also seen (Fig. 2.6d). Secretions were seen in the lumen, and protruding from individual cells. These secretory cells stained strongly with both the carbohydrate stains, Alcian blue and PAS, and were pale blue with Mallory’s trichrome suggesting the presence of a mucin.

**Vitellogenesis**

Large numbers of sperm were seen in the lumen during early vitellogenesis (Fig. 2.6b). Most were present between the folds of the mucosa, and were associated with an extracellular matrix. In some animals, a few sperm remained in the vagina during late vitellogenesis.
Figure 2.6. Vagina of *Hoplodactylus maculatus*. A: Cross section of vagina during early pregnancy showing luminal epithelium (e), the lumen (l), muscle layer (m) and connective tissue (t). H&E with Alcian Blue. Scale bar = 100 μm. B: Luminal epithelium during early vitellogenesis. Note both ciliated (c) and non-ciliated (n) cells, and the presence of sperm (arrow). SEM. Scale bar = 10 μm. C: Luminal epithelium during late vitellogenesis. TEM. Scale bar = 10 μm. D: Goblet cell during late vitellogenesis showing numerous large secretory granules (s). TEM. Scale bar = 10 μm.
Oviductal structure in a viviparous gecko

Pregnancy

Very little obvious change in structure was seen in the vagina between vitellogenesis and pregnancy. Occasional individual sperm were seen during early pregnancy.

Morphometric Data

Log transformation to achieve homogeneity of variances was required for epithelial cell height data. The height of the epithelial cell layer varied significantly among the four reproductive stages in the four regions examined (infundibulum: F(3,17)=21.90, p=0.0001; tube: F(3,16)=57.49, p=0.0001; uterus: F(3,18)=100.48, p=0.0001; vagina: F(3,18)=24.26, p=0.0001; Fig. 2.7). In all regions examined, cell height increased during vitellogenesis reaching a maximum at late vitellogenesis. Cilia length was not significantly different among the four reproductive stages in the infundibulum, tube and vagina (Fig. 2.7). Cilia length varied significantly with reproductive stage in the uterus (F(3,18)=4.12, p=0.0217), with maximum cilia length occurring during early pregnancy. Cilia height in early vitellogenic (3.3 ± 0.1 μm) and early pregnant (3.5 ± 0.02 μm) females was significantly different from all other treatment groups. There was no significant difference between cilia height in late vitellogenic (2.8 ± 0.03 μm) and late pregnant females (2.8 ± 0.1 μm), although they were significantly different from other reproductive stages (Fig. 2.7).

The number of ciliated cells that make up the epithelial layer (as a percentage of the total number of cells) changed significantly over the four reproductive stages in the infundibulum (F(3,17)=10.17, p=0.0005), uterine tube (F(3,16)=10.93, p=0.0004), and vagina (F(3,18)=23.89, p=0.0001; Fig. 2.8). The greatest percentage of ciliated cells in the epithelium occurred during pregnancy. Counts could not be made in the uterus as cell boundaries could not be delineated during pregnancy.

The approximate area of epithelium made up of ciliated cells did not vary significantly in the infundibulum, uterine tube or vagina over the four reproductive stages (Fig. 2.9). This was despite there being increased numbers of ciliated cells during pregnancy. This suggests that the ciliated and/or non-ciliated cells were increasing/decreasing in size. Note that no data were available for the infundibulum and uterine tube during early pregnancy as these two regions were not separated for analysis using electron microscopy at this stage. The uterus
Figure 2.7. Mean height of luminal epithelial cells and cilia length over four reproductive stages in four regions of the oviduct of *Hoplodactylus maculatus*. Error bars are standard errors. Numbers at the base of each bar represent sample size. Letters at the top of bars represent reproductive stages with significantly different (p<0.05) cell heights (a-d). Cilia length varies significantly only in the uterus (e-g).
Oviductal structure in a viviparous gecko

A. Infundibulum

- Cell height
- Cilia length

B. Tube

C. Uterus

D. Vagina

Early Vitellogenic
Late Vitellogenic
Early Pregnant
Late Pregnant

REPRODUCTIVE STAGE
Figure 2.9. Relative area of luminal epithelium made up of ciliated cells in *Hoplodactylus maculatus* over four reproductive stages. Error bars equal standard errors. Numbers at base of bars equal sample size. Note: data are not available for the infundibulum and uterine tube during early pregnancy. Letters at the top of bars for the uterus indicate reproductive stages that are significantly different from each other (a-d).
Figure 2.8. Percentage of ciliated cells (as a percentage of all cells) making up the luminal epithelium in the infundibulum, uterine tube and vagina of *Hoplodactylus maculatus* over four reproductive stages. Error bars are standard errors. Numbers at the base of bars represent sample size. Letters at the top of bars represent reproductive stages which are significantly different (p<0.05) within each region (a-d).
showed significant variation among the four reproductive stages ($F_{(3,17)} = 54.35$, $p=0.0001$). The area of epithelium made up by ciliated cells in the uterus reached a maximum during late vitellogenesis. This may not reflect a change in the number of ciliated cells, but instead an increase in area of the non-ciliated cells during pregnancy (Figs. 2.5a, e). Non-ciliated cells in the uterus appeared elongated laterally during pregnancy and may cover underlying blood vessels.

**DISCUSSION**

The oviduct of the viviparous gecko *Hoplodactylus maculatus* can be divided into five regions: infundibulum, uterine tube, isthmus, uterus, and vagina. This is consistent with a study concerning the oviparous gecko, *Tarentola mauritanica* (Picariello et al., 1989). In contrast, Boyd (1942) and MacAvoy (1976), who both briefly discussed oviductal structure in other populations of *H. maculatus*, reported that the oviduct has three regions: fallopian tube/infundibulum, uterus and vagina. Further examination with additional techniques, however, showed that the fallopian tube region was better separated into (1) the infundibulum (anterior), and (2) the uterine tube (posterior). The infundibulum showed a third cell type rarely seen in other regions of the oviduct (the bleb cell), and had staining properties quite distinct from the uterine tube (see below).

In the luminal epithelium of the oviduct of *Hoplodactylus maculatus*, up to three different cell types were found: (1) ciliated cells, which had nuclei situated basally, (2) microvillous non-ciliated cells, which had a variety of staining properties, and (3) bleb-like secretory cells. In most reptiles examined only two cell types are reported: ciliated and non-ciliated cells (for example: Guillette and Jones, 1985b; Guillette et al., 1989; Sarker et al., 1995). To my knowledge, bleb cells have not been reported in any other squamate species. However, bleb cells have been reported in the infundibulum of the tortoise *Gopherus polyphemus* (Palmer and Guillette, 1988) and in the uterus of the turtle *Chrysemys picta* (Abrams Motz and Callard, 1991). The function of bleb cells is unknown, but they may be involved in apocrine or merocrine secretory processes (Palmer and Guillette, 1988).

A variety of functions are attributed to the luminal epithelium. The ciliated cells presumably help maintain movement of mucus and cellular debris down the oviduct. They may also act in sperm transport and movement of the ova (Palmer
Oviductal structure in a viviparous gecko

and Guillette, 1988). Microvillous secretory cells are thought to produce mucus, which is necessary for the lubrication of the oviduct (Aitken and Solomon, 1976) and may be involved in sperm survival (Leese, 1988). The oviduct is continuous with the animal's exterior, so oviductal fluid may keep the surface epithelium moist and prevent contamination by microbes (Leese, 1988). The luminal epithelium and associated mucosal glands are also responsible for secretion of albumen and the eggshell (discussed further below). In a viviparous species, such as *Hoplodactylus maculatus*, the extra-embryonic membranes are in direct contact with the uterine luminal epithelia (to form a placenta) and must, therefore, function in physiological exchange between the embryo and the maternal tissues. The variety of staining properties and cellular distributions seen in the different oviductal regions of *H. maculatus* highlights the difference in the function(s) of each region. How these functions are achieved by the luminal epithelium is poorly understood.

The funnel-shaped ostial opening of the infundibulum initially receives the newly ovulated egg from the ovary. The epithelium of the ostial opening is predominantly ciliated, presumably to aid transport of the egg into the oviduct proper. It is unknown how the egg gets from ovary to oviduct, but it is hypothesised that the oviduct migrates to, and envelops the yolkig follicle prior to ovulation (O. Cuellar, 1970). The site of fertilisation in the reptilian oviduct is unknown, but the infundibulum is a possible candidate as fertilisation presumably must occur before any significant amount of albumen or shell membrane covers the oocyte plasma membrane.

The luminal epithelium in the infundibulum of *Hoplodactylus maculatus* contained examples of each of the three cell types listed above. The non-ciliated cells stained only inconsistently with PAS (for carbohydrate), but the presence of numerous secretory granules in these cells (usually PAS negative) confirmed secretory activity. In the oviparous lizard *Sceloporus woodi*, deposition of secretory material begins immediately upon entry of the egg into the oviduct (Palmer et al., 1993). This was indicated by an egg only halfway inside the ostium, where secretory material was already coated on the portion of the egg inside the oviduct. It was hypothesised that the secretions may include albumen proteins (Palmer et al., 1993). It may also be, simply, the secretion of mucus to aid in transport of the egg down the oviduct.
Many of the non-ciliated cells (including bleb cells) in *Hoplodactylus maculatus* showed some form of apical protrusion. Similar apical protrusions are seen in the reproductive tract (and other tissues) of mammalian species such as rats and humans (see cover of Human Reproduction, vol. 10 (5), 1995; Nikas et al., 1995). In merino ewes, for instance, apical protrusions of non-ciliated cells were numerous in the ampulla and isthmus of the Fallopian tube (Hollis et al., 1984). Nuclei were present within these protrusions during the mid-luteal and early follicular phases. Nuclei were also present within apical protrusions of *H. maculatus* during early pregnancy (when the corpus luteum would be active).

Hollis et al. (1984) reported prominent, microvilli-free projections on the apical surfaces of ciliated cells in the isthmus of merino ewes during the follicular phase of the estrous cycle. These projections contained filamentous material. The contents of these protrusions were released in an apocrine fashion into the lumen (Hollis et al., 1984). Apocrine release of the contents of similar apical protrusions was noted by Jansen and Bajpai (1982) from isthmic non-ciliated cells in the estrous rabbit. I hypothesise that the 'bleb' cells noted in *H. maculatus* resemble these protrusions and are possibly similar to those previously reported in turtles (Palmer and Guillette, 1988; Abrams Motz and Callard, 1991). Apical protrusions with and without microvilli were also reported in the ampulla and isthmus of cows (Abe and Oikawa, 1993b) and the Chinese Meishan pig (Abe and Oikawa, 1992). In these species, apical protrusions often appeared to 'pinch' off into the lumen during the luteal phase. Progesterone, which is secreted by the corpus luteum, is known to be a factor in controlling secretory activity in reproductive tract tissues of certain mammals (Leese, 1988).

Apical protrusions known as pinopodes were seen on the uterine luminal surface in humans and rats (Martel et al., 1991; Nikas et al., 1995) at the time that the uterus was receptive to implantation of the blastocyst (nidation window). The appearance of pinopodes was entirely progesterone-dependent, and the presence of estrogens induced their regression (Martel et al., 1991; Nikas et al., 1995). The pinopodes are thought to be involved in the epithelial uptake of fluid (pinocytosis) and macromolecules (endocytosis), but their precise function is not known (Nikas et al., 1995).

In mammals, as in reptiles, the nature and function of apical protrusions is not understood. Bleb cells may not be a separate cell type at all, but a stage in the activity of secretory/non-ciliated cells. This suggestion is possibly best defended by the presence of bleb-like protrusions in a variety of sizes on both ciliated and
non-ciliated cells in the infundibulum. Where whole cells appear to be pinching off into the lumen, a form of holocrine secretion may be occurring.

The uterine tube showed very different staining properties to the infundibulum. Non-ciliated cells in this region stained strongly for both PAS and Alcian blue, suggesting that a different form or combination of carbohydrates makes up the secretory granules in comparison to other oviductal regions. The uterine tube is homologous with the avian magnum, which is known to secrete albumen (Aitken and Solomon, 1976). The ultrastructural features of the uterine tube resemble those of the magnum, except that the uterine tube lacks the terminal mucous region, which in birds functions to form the 'thick layer' of egg albumen (Aitken and Solomon, 1976). In turtles and crocodilians, the thick layer of albumen which surrounds the egg at oviposition (M. Packard et al., 1988) is thought to be secreted by the uterine tube. For instance, albumen proteins were produced and secreted by the uterine tube in vitro in the turtle *Pseudemys s. scripta* (Palmer and Guillette, 1991). In lepidosaurians (squamates and tuatara), however, no albumen surrounds the egg at oviposition and the function of the uterine tube in these species is unknown. Various albumen proteins have been detected in the eggs of certain squamate species (Palmer and Guillette, 1991), but it is unknown whether these proteins were of oviductal or ovarian origin. In the lizard *Lacerta sicula*, a single albumen protein (avidin) was detected in oviductal secretions (Botte et al., 1974). Therefore, the composition, production and function of albumen in reptiles are areas in need of a great deal more research (see Palmer and Guillette, 1988). To my knowledge, no information concerning the possible secretion or composition of albumen in any member the family Gekkonidae or any viviparous reptiles is available.

The uterine tube is also a site for sperm storage (discussed further below). Halpert et al. (1982) suggested that cilia may beat in reverse during vitellogenesis to aid the movement of sperm towards the infundibulum, assuming that this is the site of fertilisation. The uterine tube is also a possible site for fertilisation as no sperm are seen above the posterior portion of the uterine tube. If this is the case, mechanisms that allow the sperm to penetrate the secretions already coating the egg must be available. The mechanism of fertilisation in reptiles is yet another area of research which needs addressing.

The isthmus is a short, muscular region between the uterine tube and the uterus. It presumably functions solely as a sphincter between the two regions. Boyd
(1942) suggested the sphincter may be an adaptation to viviparity, allowing the uterus to fit closely around the egg. However, enclosing the egg securely is likely to be equally important in an oviparous species.

The uterine region, where the embryo remains until parturition, showed the most dramatic changes over the four reproductive stages. The presence of the yolky egg and embryo during pregnancy caused the uterus to be highly stretched, in contrast to its thick-walled condition during vitellogenesis. The epithelial layer during vitellogenesis was made up of columnar cells, the non-ciliated cells of which stained positively with PAS for a form of carbohydrate, but only slightly with Alcian blue (for acid mucosubstances). This suggests a difference in secretory content of these cells compared with those of the uterine tube and vagina. This is consistent with reports concerning other reptiles (for example: *Gophorus polyphemus*, Palmer and Guillette, 1988).

During vitellogenesis, the uterus from the population of *Hoplodactylus maculatus* studied here had only a few alveolar glands scattered in the mucosa. Boyd (1942) reported the same finding for what is suggested to be a different species (Daugherty *et al.*, 1994; Hitchmough, 1997) from the *H. maculatus* complex. This situation differs from that reported for oviparous species, in which the vitellogenic uterus is greatly thickened by large numbers of uterine mucosal glands (e.g.: Christainsen, 1973; Uribe *et al.*, 1988; Guillette *et al.*, 1989; Palmer *et al.*, 1993). In oviparous species, the uterus is the region from which the eggshell membranes and calcium for the eggshell are secreted (Aitken and Solomon, 1976). The eggshell fibres are secreted by the uterine mucosal glands (Palmer *et al.*, 1993) and it is hypothesised that the calcium is secreted by the uterine luminal epithelium (Palmer and Guillette, 1988; Guillette *et al.*, 1989; Palmer *et al.*, 1993; Sarker *et al.*, 1995). In *H. maculatus*, all that remained of the eggshell was a thin shell membrane seen only during the early stages of pregnancy. This is probably a reflection of the small number of uterine mucosal glands present. Little is known of the composition of the shell membrane, although it appears fibrous in nature. The presence of relatively few mucosal glands in the uterus is a feature common to viviparous reptiles that have evolved viviparity independently of *H. maculatus* (for example: *Sceloporus aeneus*, Guillette and Jones, 1985b).

In viviparous species, a placenta is required to allow for physiological exchange between maternal and foetal tissues, including simple gas and water exchange
Oviductal structure in a viviparous gecko

(Guillette, 1993). In some species, with a more advanced placenta, nutrients are passed directly to the embryo, replacing the need for a yolk supply (for instance, *Mabuya* species, Blackburn et al., 1984). The development of the omphaloplacenta (yolk sac placenta) and chorioallantoic placenta has been described in detail in one species (undescribed) from the species complex *Hoplodactylus maculatus* (Boyd, 1942). The uterine epithelium that directly contacts the extra-embryonic membranes during pregnancy must have a function in the necessary physiological exchange. During pregnancy the epithelium was extremely thin, and there were numerous blood vessels underlying the epithelium. This suggests that the epithelium functions as a pathway for material moving from the maternal blood vessels to the embryo.

The vagina is thought to act as a sphincter (a function equivalent to the mammalian cervix), helping to retain the egg and embryo within the uterus during pregnancy. It also acts as a sperm storage region (see below) and as a birth canal. These functions are reflected in the structure of the vagina. It has deep longitudinal folds that reduce the luminal volume during vitellogenesis, but allow for expansion during parturition. It has a thick muscle layer that may act both as a sphincter and, during parturition, to help expel the embryo. The epithelium has numerous cilia, which presumably aid transport of sperm up the oviduct and of the mucus and cellular debris down during parturition. Large non-ciliated cells with large numbers of secretory granules presumably produce mucus for lubrication, as well as substances needed to maintain sperm while it is stored.

Sperm storage is a common phenomenon in reptiles (for example: snakes, Halpert et al., 1982; lizards, Shanthakumari et al., 1990; turtles, Gist and Fischer, 1993). It is hypothesised to be a mechanism allowing temporal separation of mating from fertilisation, and thus, the optimisation of male and female reproductive cycles (see Birkhead and Mollard, 1993 for discussion). In other populations within the *Hoplodactylus maculatus* species complex, copulation is believed to occur in the autumn (Whitaker, 1982; Robinson, 1985). Sperm were stored in the vagina over the winter months (MacAvoy, 1976). They then start to move up to the storage tubules in the posterior uterine tube in early spring when they were evident in the lumen of the uterus and uterine tube, and in the storage tubules themselves. Ovulation and fertilisation occurred in spring. However, the site of fertilisation is unknown.
Oviductal structure in a viviparous gecko

Sperm storage is also seen in oviparous gecko species such as *Phyllocaustus homolepidurus* (O. Cuellar, 1966). In the Australian gecko, *Phyllocaustus marmoratus*, sperm were stored over the winter months in lamellae of the vagina (King, 1977). Sperm storage areas in the vagina and infundibulum/uterine tube regions were also reported in the *Heteronotia binoci* complex (Whittier et al., 1994) and in *Lepidodactylus lugubris* (Saint Girons and Ineich, 1992). Insufficient data are available to determine whether a similar mechanism of, or location for, sperm storage is seen in gecko species in general.

Development of the oviduct in *Hoplodactylus maculatus* was seen during vitellogenesis, with epithelial cell height reaching a maximum in late vitellogenesis. The development of the oviduct is controlled by reproductive hormones. Estrogens, which are produced by the reptilian ovary during vitellogenesis, are known to promote oviductal growth in reptiles (Mead et al., 1981; Guillette, 1987; Abrams Motz and Callard, 1991). Ovariectomy causes regression of oviductal glands (Yaron, 1972; Blackburn, 1982), whereas exogenous estrogen causes gland growth and development (Yaron, 1972; Christainsen, 1973; Botte et al., 1974; Abrams Motz and Callard, 1991). Progesterone secreted by the corpora lutea is thought to be responsible for the secretory activity of epithelial cells (Palmer and Guillette, 1990; Abrams Motz and Callard, 1991; Sarker et al., 1995). In mammals such as the rabbit, epithelial cell height and secretory activity increase during estrogen dominance. Release of secretory granules occurs after coitus or progesterone administration (Leese, 1988). In rabbits, it is thought that glycoproteins are present in secretory granules found in secretory cells, their formation being controlled by estrogen and their release by progesterone (Leese, 1988). This may well be true in *H. maculatus* also. In the infundibulum, for instance, large numbers of secretory granules were seen in non-ciliated cells during vitellogenesis, when estrogen levels are presumably high. During pregnancy, when progesterone is assumed to be produced by the corpus luteum, many apical protrusions were noted, and often whole cells appeared to be pinching off into the lumen. In reptiles, as with other vertebrates, progesterone is secreted by the corpus luteum. However, whether progesterone is involved with the secretory activity of the reptilian oviduct is not known. In addition, progesterone from the corpus luteum acts in the maintenance of pregnancy (Xavier, 1987). In oviparous reptiles, the length of egg retention is associated with the length of time the corpus luteum is active. This association does not necessarily hold for viviparous species (Guillette, 1987).
In conclusion, the general structure of the oviduct in *Hoplodactylus maculatus* resembles that described in other reptilian species. *H. maculatus* shares a common characteristic with other viviparous squamates, in that very few glands are present in the uterine mucosa, resulting in a greatly reduced eggshell. Changes were seen in the oviduct of *H. maculatus* over the reproductive season, with development of the oviduct occurring during vitellogenesis in preparation for pregnancy. These changes were most pronounced in the uterine region. This is not surprising considering the tissue must contain a developing embryo during gestation. Ultrastructural features, such as bleb cells and other apical protrusions, seen during this study highlight the lack of understanding still surrounding the functions and method of action in the combination of tissues which make up the reptilian oviduct.
Chapter Three

Oviductal structure in three species of gekkonid lizard exhibiting variations in parity mode and eggshell structure

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ABSTRACT.

Oviductal structure was analysed in three species of gekkonid lizard exhibiting variation in parity mode and eggshell structure: *Hemidactylus turcicus* (oviparous) which produces a hard, calcareous eggshell; *Saltuarius wyberba* (oviparous) which produces a soft, parchment-like eggshell; and *Hoplodactylus maculatus* (viviparous). All females examined were in late vitellogenesis. Oviducts were analysed using light, scanning electron and transmission electron microscopy. The oviduct was separated into five regions: infundibulum, uterine tube, isthmus, uterus and vagina. The uterus exhibited differences among species that were directly attributable to parity mode. *H. turcicus* and *S. wyberba* (both oviparous) had numerous uterine shell glands; *H. maculatus* (viviparous) had very few. The uterus also exhibited differences between the two oviparous species (*H. turcicus* and *S. wyberba*) which may be related to the type of eggshell produced. Variations were noted in the staining properties of the uterine glandular and epithelial cells and in the quantity of secretory granules present. The structure of the infundibulum, uterine tube, isthmus and vagina also differed among species, but differences could not be directly related to parity mode or eggshell structure. Instead, the differences may be related to how prepared the oviduct is for ovulation in individuals analysed from the different species. This study confirms, in the Gekkonidae, aspects of oviductal structure that have been associated with parity mode in other squamate taxa. The results highlight the limited knowledge concerning the changes in oviductal structure during the period immediately prior to ovulation and egg shelling.
INTRODUCTION

Previous studies of lizards show that differences in oviductal function associated with differing parity modes are reflected in oviductal structure. For instance, the fibres of the eggshell produced by oviparous reptile species are thought to be secreted by uterine mucosal (shell) glands (Palmer et al., 1993). Viviparous species, in which the mothers retain their embryos until development is complete (Guillette, 1993), do not require an eggshell; therefore, fewer shell glands are expected. This is consistent with the pattern seen in reptiles examined to date: oviparous species have numerous shell glands in the uterus, whereas viviparous species have very few (Christainsen, 1973; Guillette and Jones, 1985b; Uribe et al., 1988; Guillette et al., 1989; Guillette, 1992; Palmer et al., 1993).

Considerable variation exists in eggshell structure between reptilian species (M. Packard and DeMarco, 1991), but as yet, little attention has been paid to how the oviduct may vary between species producing different eggshell types. Guillette et al. (1989) noted differences in the type of uterine shell gland, and in the materials produced by these glands, in two phylogenetically distinct lizard species, Crotophytus collaris and Eumeces obsoletus.

In this study, I compared the structure and ultrastructure of the oviduct from several species of gekkonid lizard exhibiting different parity modes. I chose species from the Gekkonidae as this family contains species that are oviparous (egg-laying) and viviparous. Additionally, some oviparous geckos produce a hard, calcareous eggshell (subfamilies Gekkoninae and Sphaerodactylinae), whereas some produce a soft, parchment-like eggshell (subfamilies Diplodactylinae and Eublepharinae; Bustard, 1968). Viviparity is thought to have evolved in the Gekkonidae independently of its evolution in other families (Blackburn, 1982; Shine, 1985). No previous comparisons of oviduct structure have been made between gekkonids of different parity modes or between oviparous geckos producing different eggshell types.

The species analysed in this study were: Hemidactylus turcicus (Gekkoninae), which is oviparous and lays a hard, calcareous eggshell (M. Packard et al., 1982; M. Packard and Hirsch, 1986, 1989); Saltuarius wyberba (Carphodactylini, Diplodactylinae), which is also oviparous, but is thought to lay a soft, parchment-like eggshell (Couper et al., 1993); and Hoplodactylus maculatus (Carphodactylini,
Diplodactylinae), which is viviparous. Additional tissues from *Hoplodactylus duvaucelii* (viviparous, Carphodactylini) were also examined.

Oviductal tissues were obtained from females in late vitellogenic condition. During vitellogenesis, the oviduct undergoes development in preparation for gravidity or pregnancy (Mead *et al.*, 1981; Abrams Motz and Callard, 1991). Thus, structures associated with shell production should be seen at this stage. We expected to see numerous shell glands in the uterus of the oviparous species and fewer in the viviparous species. Differences in the structure of uterine glands and epithelial cells between the two oviparous species were predicted due to differing eggshell structures. Few differences in the structure of other regions (infundibulum, uterine tube, isthmus and vagina) were expected.

**MATERIALS AND METHODS**

*Animal Collection*

Oviducts from vitellogenic females of three gecko species were analysed in detail. *Hemidactylus turcicus* (oviparous with hard eggshell) were captured off building walls and windows in Gainesville, Florida, USA, in May, 1996 (n=4). *S. wyberba* (oviparous with soft eggshell) were captured from large rocks in open woodland in Girroween National Park, Queensland, Australia, in October, 1995 (n=3; Scientific Purposes Permit W0/000989/95/SAA, carcasses are lodged at the Queensland Museum, Brisbane, Australia). *Hoplodactylus maculatus* (viviparous) were collected from under loose rock slabs on schist outcrops near Macraes Flat in Central Otago, New Zealand in September, 1995 (n=5, Chapter 2). Ovulation occurs in September/October in this population of *H. maculatus* (J. Girling, personal observation). The oviducts from *H. maculatus* in vitellogenic and other reproductive conditions have been described in a separate study (Chapter 2).

Reproductive condition was initially determined by gentle palpation of the abdomen (Cree and Guillette, 1995) and confirmed upon dissection. All females collected were in late vitellogenic condition as indicated by the presence of a large, yolky follicle in each ovary (*H. turcicus*: follicle diameter 4-6 mm, snout-vent length (SVL) 51-58 mm; *S. wyberba*: follicle diameter 11-14 mm, SVL 98-108 mm; *H. maculatus*: follicle diameter 9-11 mm, SVL 62-76 mm). Rose and Barbour
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(1968) reported ovulation occurring when follicles reached 8-8.5 mm diameter in H. turcicus in the southern United States. In another population of H. maculatus from Central Otago, follicle diameter was 6-10 mm in late vitellogenic females (Cree and Guillette, 1995). H. turcicus and H. maculatus females were killed by decapitation (swift blow to the head followed by severance of the spinal column). S. wyberba females were killed by a lethal injection of Nembutal (pentobarbitone sodium).

An oviduct from a single preserved specimen (fixed in Bouins, stored in 70% ethanol) of Hoplodactylus duvaucelii (viviparous), which was initially captured from North Brother Island, New Zealand, in November, 1990, was examined using light microscopy only. The animal was collected primarily for studies on systematics under a Department of Conservation permit to C. H. Daugherty, Victoria University of Wellington. This female was also in late vitellogenesis (follicle diameters: 16-17 mm, SVL: 110 mm). Another female collected on the same date had recently ovulated, consistent with ovulation occurring during October-November as in other populations of this species (Cree, 1994 and pers. comm.).

Histology

One oviduct from each female from all four species was preserved in Bouins fixative and then stored in 70% ethanol. The oviducts from H. turcicus and H. maculatus were processed in situ to retain shape and proximity to other tissues. Oviducts from S. wyberba and H. duvaucelii were dissected out of the body as carcasses were required for preservation. The tissues were washed and dehydrated in ethanol (70%, three x 100%), cleared in xylene, embedded in paraffin, serially sectioned (6-8 μm) and mounted on glass slides. Three slides from every 10 were stained to visualise changes along the entire length of the oviduct. One of every 10 slides was stained with Lillie-Mayer Haematoxylin and Eosin (H&E, general histology) with an Alcian Blue (pH 2.5) counterstain (Humason, 1979). At this pH, Alcian Blue stains both carboxylated and sulfated acid mucosubstances (forms of carbohydrate, Humason, 1979; Sheehan and Hrapchak, 1980. The second of 10 slides was stained with Mallory’s Trichrome (general histology and connective tissue), and the third with Periodic Acid-Schiff’s Reagent (PAS) with a Fast Green counterstain (Humason, 1979). Periodic Acid stains a variety of carbohydrates, including some carbohydrate-protein and carbohydrate-lipid compounds (Humason, 1979; Sheehan and Hrapchak, 1980).
Representative slides from each region (from each species analysed) were subjected to the saliva (diastase) test prior to PAS treatment (Humason, 1979; Sheehan and Hrapchak, 1980). No differences between the slides treated with or without the saliva test were noted, therefore eliminating glycogen as the staining product.

**Scanning and Transmission Electron Microscopy**

The second oviduct from *H. turcicus, S. wyberba* and *H. maculatus* was dissected out of the body cavity and cut into different regions with a sharp razor blade. Regions visible with the naked eye were separated: infundibulum, uterine tube, uterus and vagina. The isthmus (a very short region) was generally destroyed by cutting during preparation for electron microscopy and was only observed using light microscopy.

Each tissue region was cut into small pieces (approximately 1 mm²) and separated for use in scanning and transmission electron microscopy. Tissues were initially preserved in 2.5% glutaraldehyde in phosphate buffer for 2.5 h, followed by post-fixation in 1% osmium tetroxide/1.5% potassium ferrocyanide in cacodylate buffer for 1 h. Tissues for scanning electron microscopy (SEM) were dehydrated down an ethanol series and critical-point-dried in liquid CO₂. The samples were then mounted and coated with gold palladium and viewed using a Cambridge S360 microscope at 20 kV. Tissues for transmission electron microscopy (TEM) were dehydrated down an ethanol series and infiltrated with agar resin. Semi-thin and ultra-thin sections were cut using an Ultracut E (Leica Instruments Pty Ltd, Australia). Samples were viewed on a Philips EM410 microscope (Holland).

**RESULTS**

**General Description**

The abdominal cavity of all females was dominated by two large, yolky follicles, one in each ovary. One ovary, generally the left, was often positioned more anteriorly than the other. In all species examined, the oviducts lay along the dorsal body wall. In both *Hemidactylus turcicus* and *Saltuarius wyberba*, the oviduct and surrounding mesotubarium were creamy white. In *Hoplodactylus maculatus* (Chapter 2) and *Hoplodactylus duvaucelii* (to a lesser extent) the
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mesentery and oviduct were black due to the presence of dispersed melanin in melanocytes.

In each of the four species described, five regions of the oviduct were recognised using light microscopy: infundibulum, uterine tube, isthmus, uterus and vagina (Fig. 3.1a). The epithelial layer lined the lumen of the oviduct and contained up to three cell types: ciliated, microvillous non-ciliated, and bleb-like non-ciliated (bleb) cells. Ciliated cells in all regions were dominated by a large nucleus. The cytoplasm of ciliated cells contained large numbers of mitochondria, especially at the apical end of the cells.

The oviduct was differentiated into several layers in cross section (Fig. 3.1b): 1. the mucosa: made up of an epithelial layer of ciliated and/or non-ciliated cells lining the lumen of the oviduct and an underlying lamina propria consisting of connective tissue, blood vessels and glands (if present); 2. the muscularis: consisted of inner circular and/or outer longitudinal muscle layers; and 3. the serosa: outermost layer continuous with the peritoneum. These layers differed in thickness and function depending on which oviductal region they were from. Individual regions of the oviduct are described in more detail below.

**Infundibulum**

In all four species examined, the infundibulum (Figs. 3.2, 3.3) was a thin-walled region with an indistinct muscle layer. In *H. turcicus*, the vast majority of non-ciliated cells seemed to be protruding into the lumen (Figs. 3.2a, 3.3a,b). The nuclei were positioned within the protrusion, at the apical end of non-ciliated cells, and were surrounded by a thin layer of cytoplasm which contained some small secretory granules (Figs. 3.2a, 3.3b). The surface of these cells was usually irregular (Figs. 3.3a,b). The number of microvilli on the apical protrusions varied. Some cells had numerous microvilli, but others had none. At the posterior part of the infundibulum, apical tips of some of the epithelial cells stained positively with PAS for carbohydrate. The staining abundance decreased anteriorly. There was no positive PAS staining at the ostium. Very few ciliated cells were visible except near the opening of the ostium. Posterior to the ostium, ciliated cells were hidden among the protruding non-ciliated cells making them very difficult to see with the SEM (Fig. 3.3a).
**Figure 3.1.** A: Gross morphology of gekkonid oviduct drawn from dissection of *Hoplodactylus maculatus*. a: infundibulum, b: uterine tube, c: isthmus, d: uterus, e: vagina. Bar = 5 mm. B: Schematic diagram of cross section through oviduct illustrating different tissue types. a: epithelium, b: shell gland, c: connective tissue, d: circular muscle, e: longitudinal muscle, f: serosa (mucosa = a+b+c, muscularis = d+e). Bar = 100 μm.
Figure 3.2. Light micrographs of the infundibulum from A: *Hemidactylus turcicus*, B: *Saltuarius wyberba*, and C: *Hoplodactylus maculatus*. Arrow indicates ciliated cell. e: epithelium, l: lumen, m: muscularis, s: serosa. Bars: 50 µm
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Figure 3.3. Electron micrographs from the infundibulum. A: Scanning electron micrograph and B: transmission electron micrograph of epithelial cells from Hemidactylus turcicus. Note non-ciliated cells protruding into the oviductal lumen. C: Scanning electron micrograph of bleb-like non-ciliated cells and D: transmission electron micrograph of standard epithelial cells in Saltuarius wyberba E: Scanning electron micrograph and F: transmission electron micrograph of epithelial cells from Hoplodactylus maculatus. Enlargements of figures G: 3.3b, H: 3.3d and I: 3.3f (see following pages). b: bleb-like non-ciliated cell, c: ciliated cell, l: lumen, k: nucleus, n: non-ciliated cell, o: secretory granules. Bars: 10 μm
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Oviductal structure in three species of gecko
The infundibulum differs in *S. wyberba* and *H. maculatus* (Figs. 3.2, 3.3) and *H. duvaucelii* (Fig. 3.4a). The epithelium in these species also contained both ciliated and micro-villous non-ciliated cells, but in different proportions to *H. turcicus*. At the lip of the ostium, the epithelium was predominantly ciliated, becoming more non-ciliated posteriorly. The cilia on ciliated cells were long and easy to distinguish using light microscopy (Figs. 3.2b,c). Microvilli on non-ciliated cells were numerous and prominent (Fig. 3.3e). Non-ciliated cells did not stain with Alcian Blue for acid mucosubstances, but were PAS (carbohydrate) positive, mainly at the apical end of the cells. The positive PAS staining corresponded with numerous electron dense secretory granules present at the apical end of these cells (Figs. 3.3d,f). Some apical protrusions and bleb cells were seen (Fig. 3.3c). The nuclei in these cells were situated basally. In *H. maculatus*, the non-ciliated and ciliated cells tended to be of different heights, giving the epithelium an irregular appearance (Fig. 3.3e,f).

**Uterine tube**

The uterine tube was very similar in all species examined (Fig. 3.4b, 3.5, 3.6). The muscle layer was thin, but distinct from other layers, although circular muscle could not be distinguished from longitudinal muscle layers. In the posterior part of the uterine tube, the mucosa formed folds. Glands could be found at the base of these mucosal folds (Figs. 3.5, 3.6b). The cells of these glands did not stain with either the PAS or Alcian Blue procedures, but did contain numerous secretory granules of varying electron densities (Fig. 3.6f). These glands were only found at the posterior end of the uterine tube. In *H. duvaucelii*, mucosal glands were more numerous than in other species examined (Fig. 3.4b). In *H. maculatus*, sperm were present in the most posterior glands, just above the isthmus, and sperm could also be seen on the surface of the epithelium (Fig. 3.6e).

The epithelium was made up of both ciliated and microvillous non-ciliated cells (Figs. 3.5, 3.6c,e). Ciliated cells had long, distinctive cilia (Figs. 3.6c,e). The microvillous non-ciliated cells, which predominated, stained strongly with Alcian Blue for acid mucosubstances and with PAS for carbohydrate. Staining was seen throughout the cell. Staining corresponded to the presence of large secretory granules of varying electron densities (Fig. 3.6d). Granules were found throughout the cell, dominating the cytoplasm. The granules were interspersed with other organelles including numerous mitochondria. They also stained blue with Mallory's Trichrome suggesting some sort of mucin. In *H. maculatus*, the
Figure 3.4. Light micrographs from the oviduct of *Hoplodactylus duvaucelii*: A: infundibulum, B: uterine tube, C: isthmus, D: uterus and E: vagina. e: epithelium, g: gland, l: lumen, m: muscle, s: serosa, z: lamina propria. Bars: 50 μm
Figure 3.5. Light micrographs of the uterine tube from A: *Hemidactylus turcicus*, B: *Saltuarius wyberba* and C: *Hoplodactylus maculatus*. g: gland, l: lumen, m: muscle, s: serosa, z: lamina propria. Bars: 50 μm
Figure 3.6. Electron micrographs of the uterine tube. A: Scanning electron micrograph and B: transmission electron micrograph of the epithelium from *Hemidactylus turcicus*. Note glandular material. C: Scanning electron micrograph and D: transmission electron micrograph of epithelium from *Saltuarius wyberba* E: Scanning electron micrograph of epithelium (note arrow indicating sperm) and F: transmission electron micrograph of gland cells from *Hoplodactylus maculatus*. Note the secretory granules of varying electron densities. G: Enlargement of figure 3.6a (see over page). c: ciliated cell, g: gland, k: nucleus, l: lumen, n: non-ciliated cell, o: secretory granule, z: *lamina propria*. 
Bars: 10 μm
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staining tended to be concentrated at the apical tip of the cell. In *H. turcicus*, some areas of epithelium were predominantly ciliated. These areas were very patchy along the uterine tube (Figs. 3.6a,b).

*Isthmus*

The isthmus was intermediate in appearance between the uterine tube and the uterus (Figs. 3.4c, 3.7). In both oviparous species (*H. turcicus* and *S. wyberba*), the isthmus region was glandular (Figs. 3.7a,b), whereas only very few glands were present in the isthmus of *H. maculatus* and *H. duvaucelii* (Figs. 3.4c, 3.7c). *H. duvaucelii*, however, had more glands than *H. maculatus*. The major difference between the isthmus when compared to the uterus and uterine tube was that non-ciliated cells showed no staining with either of the carbohydrate stains used (Alcian Blue or PAS). In *H. turcicus* and *H. maculatus*, the region was very short and easily missed. The isthmus may be longer in *S. wyberba* in comparison to the other species. Relative length of the isthmus, however, was not measured. Glands of the isthmus in *S. wyberba* stained less intensely than glands in the uterus suggesting that contents of gland cells differed between regions.

*Uterus*

The uterine region showed the most obvious differences among the species (Figs. 3.4d, 3.8, 3.9). In all species, there was a distinctive muscle layer with both inner circular and outer longitudinal layers (Figs. 3.8a,c,e). Capillaries were quite common within the muscle layer.

In the oviparous species *H. turcicus*, the mucosa was packed with lightly staining glands (Fig. 3.8a). The gland cells were arranged in a generally circular arrangement around a central lumen. The gland cells contained granular material, even at the light microscopy (LM) level, and did not stain with either of the carbohydrate stains (Alcian Blue or PAS). Gland cell nuclei were situated at the basal portion of the cells (Fig. 3.8b). Within the cytoplasm were large secretory granules of a uniform electron density. The granules were not densely packed, and numerous mitochondria were also present within the cytoplasm (Fig. 3.8b). Microvilli were present on the apical surface of cells lining the lumen of the glands.
Figure 3.7. Light micrographs of the isthmus from A: *Hemidactylus turcicus*, B: *Saltuarius wyberba* and C: *Hoplodactylus maculatus*. e: epithelium, g: gland, l: lumen. Bars: 50 μm.
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Figure 3.9. Electron micrographs of uterine epithelium. A; B; C: Scanning electron micrographs and D: transmission electron micrograph from *Hemidactylus turcicus*. Note varied structure of non-ciliated cells. Mitochondria are large and distinct (white arrow). Low electron dense granules can be found at the basal end of the cell (black arrow). E: Scanning electron micrograph and F: transmission electron micrograph from *Saltuarius wyberba* Cells contain numerous vesicles (large black arrow). G: Scanning electron micrograph and H: transmission electron micrograph from *Hoplodactylus maculatus*. Enlargement of figures I: 3.9d, J: 3.9f and K: 3.9h (see following pages). c: ciliated cell, k: nucleus, l: lumen, n: non-ciliated cell, o: secretory granules. Bars: a-b = 1 μm, c-k = 5 μm.
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Oviductal structure in three species of gecko
The stratified epithelial cells lining the uterus of *H. turcicus* were columnar and densely packed (Fig. 3.9d). Nuclei within the cells were elongated. The epithelium was composed predominantly of non-ciliated cells which stained with both the PAS (carbohydrate) and Alcian Blue (acid mucosubstances) procedures. Staining corresponded to the presence of secretory granules observed at the TEM level. The extent of staining varied among individual *H. turcicus*, ranging from staining throughout the cell to a fine layer at the apical surface. These cells had a varied appearance when viewed using SEM. Some had an even apical surface (Fig. 3.9a), others had a sunken centre (Fig. 3.9b), whereas others were protruding (Fig. 3.9c), the protrusion being either full or sunken. Some cells appeared to be budding off into the lumen with most of the cytoplasm (and organelles) present within the protrusion. Cells adjacent to the lamina propria contained several large granules of low electron density at the basal region of the cell (Fig. 3.9d).

A very similar arrangement was seen in oviparous *S. wyberba* (Fig. 3.8c). However, there were several differences. The gland cells were filled with very intensely staining granular material. As in *H. turcicus*, gland cells contained large secretory granules (Fig. 3.8d). The granules appeared more densely packed than in *H. turcicus*. Mitochondria were also present, but were less conspicuous than in *H. turcicus*. The uterine epithelium was columnar and appeared stratified (Figs. 3.9e,f). The epithelial cells were not densely packed as in *H. turcicus*; individual cells were easily distinguished from each other. Non-ciliated cells in the epithelium stained positively with the Alcian Blue stain for acid mucosubstances, predominantly at the apical tip of the cell. Patches of positive staining were inconsistently seen in other cell areas. The apical end of the non-ciliated cells contained a mix of secretory granules and vesicles (Fig. 3.9f). The vesicles seen in the uterus of *S. wyberba* were not noted in the uterine epithelium of the other species analysed. Ciliated cells were packed with mitochondria and vesicles (as in non-ciliated cells), some of which contained quite large pieces of material (Fig. 3.9f). Some patches of golgi apparatus were visible, but no developing granules were noted. Microvilli were present on ciliated cells (Fig. 3.9e).

In *H. maculatus* and *H. duvaucelii* (viviparous), only occasional glands were seen in the muccsa (Figs. 3.8e, 3.4d). The cells of the glands superficially resembled epithelial cells, although they did not stain with either of the carbohydrate stains used (Alcian Blue or PAS). Using the TEM, it could be seen
that gland cells in *H. maculatus* contained large numbers of secretory granules (Fig. 3.8f). The granules from *H. maculatus* appeared smaller and more electron dense than those in either *H. turcicus* or *S. wyberba*. Uterine tissues from *H. dufaueilii* were not analysed using TEM. The mucosa itself was made up of a layer of connective tissue, and was more folded in *H. maculatus* (Fig. 3.8e) than in the two oviparous species (Figs. 3.8a,c).

The uterine epithelium of *H. maculatus* was made up of ciliated and micro-villous non-ciliated cells (Figs. 3.9g,h). The cilia were bent down over the surface of the cells, all lying in one direction (Fig. 3.9g). The non-ciliated cells stained slightly (very diffuse) with the Alcian Blue stain for acid mucosubstances, but strongly with the PAS for carbohydrate, and staining corresponded to large numbers of secretory granules of varying electron densities (Fig. 3.9h). Occasional sperm could be seen on the surface of the epithelium.

**Vagina**

The most anterior part of the vagina had a thick muscle layer (predominantly circular muscle, Figs. 3.4e, 3.10). This muscle layer, and the distinctive finger-like mucosal folds of the vagina, became reduced in size posteriorly. In all three species, the epithelium was predominantly ciliated in the vagina (Figs. 3.11a,c,e).

In *H. turcicus*, the epithelium was low, almost cuboidal (Fig. 3.10a), and non-ciliated cells had only slight PAS (carbohydrate) and Alcian Blue (acid mucosubstances) positive staining at the apical surface. Non-ciliated cells often formed oddly-shaped apical protrusions which appeared to be sloughing off into the lumen (Figs. 3.11a,b). The contents of these protrusions tended to be of uniform consistency, i.e. distinctive organelles did not appear to be present. Very few, if any, secretory granules were seen in non-ciliated cells of *H. turcicus* (Fig. 3.11b). Mitochondria were present in all cells, some being very large with obvious cristae. Distinctive areas of golgi apparatus were present in many ciliated cells. Numerous sperm were present (Fig. 3.11a).

In *S. wyberba*, the non-ciliated cells stained only lightly with the Alcian Blue stain for acid mucosubstances and very slightly (diffuse) with PAS for carbohydrate. Secretory granules of low electron density were present in non-ciliated cells (Fig. 3.11d). Numerous sperm were present (Fig. 3.11c).
Figure 3.10. Light micrographs of the vagina from A: *Hemidactylus turcicus*, B: *Saltuarius wyberba* and C: *Hoplodactylus maculatus*. e: epithelium, l: lumen, m: muscle, z: lamina propria. Bars: 50 μm.
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Oviductal structure in three species of gecko
In *H. maculatus* and *H. duvaucelii*, the non-ciliated cells stained with both Alcian Blue (acid mucosubstances) and PAS (carbohydrate) stains. Some of the non-ciliated cells in *H. maculatus* contained relatively small secretory granules of varying electron densities at the apical edge of the cell, or were packed with large uniformly electron dense granules (Fig. 3.11f). Some sperm were present in the vagina of *H. maculatus*, but were not seen in that of *H. duvaucelii*.

**DISCUSSION**

The function of the oviduct in reptiles is intimately associated with parity mode. It is reasonable to expect, therefore, that differences in the function of the oviduct will be reflected by differences in the structure of the oviduct. Differences in oviductal structure are apparent in the results seen in this study, as well as those reported in the literature.

Alveolar glands found in the uterine mucosa (shell glands) are thought to be responsible for secretion of the fibrous component (eggshell membrane) of the eggshell (Guillette et al., 1989; Palmer et al., 1993). Previous studies in lizard families other than the Gekkonidae have noted that shell glands are numerous in oviparous species (Christainsen, 1973; Uribe et al., 1988; Guillette et al., 1989; Palmer et al., 1993), but infrequent in viviparous species (Guillette and Jones, 1985b). The findings of this study for several members of the Gekkonidae are consistent with these observations. The two oviparous species, *Hemidactylus turcicus* and *Saltuarius wyberba*, had numerous uterine shell glands, unlike *Hoplodactylus maculatus* and *Hoplodactylus duvaucelii*, where only occasional shell glands were noted.

Oviparous species can be divided into groups based on eggshell type. A reptilian eggshell is composed of several layers (M. Packard et al., 1982; M. Packard and DeMarco, 1991): an inorganic, calcareous layer with an underlying fibrous, organic layer (shell membrane). These layers, however, vary in thickness and structure among different reptilian species (M. Packard et al., 1982; M. Packard and DeMarco, 1991). Within the family Gekkonidae, different eggshell types are produced. All species from the subfamilies Sphaerodactylinae and Gekkoninae (entirely oviparous), including *H. turcicus* (Gekkoninae, M. Packard et al., 1982; M. Packard and Hirsch, 1986), produce heavily calcified eggshells (Bustard, 1968; Werner, 1972; Shine, 1985). However, oviparous species from the subfamilies
Eublepharinae and Diplodactylinae produce parchment-like eggshells (Bustard, 1968; Werner, 1972). To our knowledge, the eggshell of *S. wyberba* (Diplodactylinae) has not been described. However, other leaf-tailed gecko species (*Saltuarius* and *Phyllurus*) from the Diplodactylinae produce soft-shelled eggs (Couper *et al.*, 1993).

In this study, the shell glands of *S. wyberba* stained more intensely than those of *H. turcicus* and appeared to be more densely packed with secretory granules. Secretory granules were also seen in the relatively few shell glands noted in *H. maculatus*. These granules were smaller and more electron dense than those in the oviparous species examined. These results suggest that there could be differences in the quantity and type of material produced by species producing different types of eggshells or shell membranes (a transient shell membrane is produced by *H. maculatus*, Chapter 2).

The calcareous component of the eggshell is thought to be secreted by the uterine epithelial layer (Guillette *et al.*, 1989; Palmer *et al.*, 1993). Cellular changes were noted in the uterine mucosa of the oviparous lizard, *Sceloporus woodi*, during the period of calcium deposition post-ovulation (Palmer *et al.*, 1993). Changes noted included cell hypertrophy with the microvilli becoming less pronounced as the apical membranes became distended (Palmer *et al.*, 1993). In the turtle, *Lissemys punctata punctata*, most of the uterine epithelial cells stained positively for calcium during gravidity (Sarker *et al.*, 1995).

As the gecko species analysed in this study had different parity modes and eggshell types, it was expected that the epithelial layer would show structural differences which could be associated with the differing needs for eggshell calcium. The epithelial layer certainly differed among the three species examined in detail. For instance, the epithelium was extremely variable in *H. turcicus*, and there were vesicles present in the epithelial cells of *S. wyberba*. The differences in structure, however, could not easily be related to possible differences in function, specifically calcium secretion. It is possible that changes associated with calcium secretion would not be seen until after ovulation when egg shelling is occurring. More specialised techniques, such as specific histochemical stains for calcium ions, confocal microscopy, or use of labelled calcium ions *in situ*, should be considered to more fully elucidate the process of calcium secretion for eggshell synthesis in reptilian species.
As an initial comparison of oviductal structure between gekkonid species with varying eggshell types, this study has provided important qualitative information. In future studies which relate oviductal structure to eggshell type, it will be necessary to determine the relative and absolute amounts of organic versus inorganic material in the eggshell, the chemical composition of the organic component of the eggshell, and the chemical composition of secretory material in the uterine shell glands and the epithelium of the species of interest.

Differences among species which were not obviously related to parity mode were also detected. These included differences in the infundibulum, uterine tube, isthmus and vagina which will be discussed below.

During vitellogenesis, the oviduct migrates towards the ovary where the infundibulum envelopes the follicle prior to it being ovulated (O. Cuellar, 1970). Differences were noted in the structure of the infundibulum among the gecko species examined. The epithelial cells of *H. turcicus* appeared to be in the process of budding off into the lumen. The non-ciliated cells protrude into the lumen and the cell’s nucleus was within the protrusion. Protruding cells were not observed in the infundibulum of the other species analysed.

To explain the different patterns seen among the species analysed, the role of steroid hormones will have to be considered. Estrogen is known to be secreted by the reptilian ovary during vitellogenesis (Mead *et al.*, 1981; Abrams Motz and Callard, 1991), stimulating the development and hypertrophy of the oviduct. In addition, plasma progesterone levels rise around the time of ovulation (Chan *et al.*, 1973; Licht, 1982; Xavier, 1982; Sarker *et al.*, 1995; A. Cree, J. E. Girling and J. Rock, personal observation for *H. maculatus*), presumably due to the formation and presence of a functional corpus luteum (see I. Callard *et al.*, 1992 for discussion). Progesterone has been implicated in several aspects of control in the reptilian oviduct, including the induction of avidin (egg-white protein) production (Botte and Basile, 1974; Botte and Granata, 1977; Yaron, 1985; Guillette *et al.*, 1991a; I. Callard *et al.*, 1992). In mammalian species, progesterone is known to stimulate the production and release of various proteins (Roberts and Bazer, 1988) and to cause cell atrophy, de-differentiation and apoptosis (Greenwald, 1969; Bareither and Verhage, 1981).

I hypothesise that an increase in plasma progesterone prior to ovulation causes the epithelium of the infundibulum to become secretory, as well as causing
apoptosis to begin in the non-ciliated cells. If this is the case, it suggests the H. turcicus females were closer to ovulation than the other species examined. Additional ultrastructural information from the ovary and oviduct prior to, and over, the period of ovulation, and measurements of plasma progesterone, would be required to confirm this suggestion.

Posterior to the infundibulum lies the uterine tube. The uterine tube had a similar structure in all species examined. The descriptions here correspond to those for the gecko Tarentola m. mauritanica (Picariello et al., 1989) and other squamates (Guillette et al., 1989; Guillette, 1992). In turtles and crocodilians, the uterine tube is believed to be responsible for secretion of the albumen layer (Palmer and Guillette, 1991). In the eggs of squamates, however, no albumen layer has been found (Cordeno-López and Morales, 1995). This suggests the primary function of the numerous non-ciliated cells (which contained abundant secretory granules) in the uterine tube is mucus secretion.

The major difference in the uterine tube among species analysed in this study was the presence or absence of sperm in glands formed at the base of mucosal folds. At the reproductive stage analysed in this study (late vitellogenesis), sperm were noted only in H. maculatus. This suggests there is variation in the timing of copulation among different gecko species. In male H. turcicus, the epididymides are packed with mature sperm from April to July (spring/summer, Rose and Barbour, 1968). Copulation was observed in a pair of geckos in June (Rose and Barbour, 1968). Females in this study were collected in May and sperm were noted in the vagina, but not the uterine tube, which suggests recent copulation. In H. maculatus, mating is thought to occur in autumn when females are in the early stages of vitellogenesis (Whitaker, 1982; Robinson, 1985). Females in this study were collected in spring and some sperm were present in the vagina, but most were in the uterine tube. This is consistent with an autumn mating and sperm being stored over the winter before transport to the uterine tube. In the gecko, Hemidactylus frenatus, collected in Hawaii, sperm can be found stored in the uterine tube (termed the transition zone by the authors) regardless of the ovarian condition of the female (Murphy-Walker and Haley, 1996).

The isthmus appears to be a transition zone between the uterine tube and the uterus, the structure of the isthmus being intermediate between the two regions. Thus, in both H. turcicus and S. wyberba, the isthmus contains mucosal glands,
Oviductal structure in three species of gecko

whereas very few glands are seen in the isthmus of *H. maculatus*. The major difference between the isthmus in comparison to the uterine tube and uterus in all three species examined was the lack of carbohydrate staining in the epithelium. In the gecko *T. mauritanica* (Picariello *et al.*, 1989), however, the non-ciliated cells of the isthmus stain strongly for carbohydrate with both Alcian Blue and PAS.

The function of the isthmus is unknown. It presumably acts as a sphincter, helping to maintain the egg within the uterus. The suggestion by Boyd (1942), that the sphincter is an adaptation to viviparity is negated by the presence of an isthmus in all three gekkonid species analysed in this study, including the two oviparous examples.

Although not measured, the isthmus in *S. wyberba* appeared proportionally longer than in *H. turcicus, H. maculatus* or *H. duvaucelii*, in which the isthmus was very short and easily missed. As the isthmus was not examined using electron microscopy, it could not be confirmed whether gland cells contained secretory granules. However, in *S. wyberba*, the difference in staining intensity of glands between the isthmus and uterus suggests a difference in content and function. It is unknown whether the mucosal glands of the isthmus contribute to the shell membrane in any of the species analysed.

The most posterior region of the reptilian oviduct is the vagina. The vagina presumably acts as a sphincter during pregnancy, helping to maintain the eggs or embryos within the uterus (Perkins and Palmer, 1996). The vagina has a large muscle layer which would be required during parturition, helping to expel the egg or embryo. No differences were predicted among the geckos exhibiting different parity modes. However, although the basic structure was similar, differences in the epithelial layer were noted, specifically the carbohydrate staining properties of epithelial cells. These differences may relate to one species analysed being slightly closer to ovulation than the others, as discussed when considering differences in the infundibulum. An increase in progesterone may have caused the apical protrusions that appear to be sloughing off into the lumen in *H. turcicus*.

In summary, the gross morphology of the oviduct was similar in all the gecko species analysed in this study. Structures described corresponded, in general, with data provided in the literature concerning other squamate species. However,
differences in uterine structure among geckos with differing parity modes and eggshell types were found. These included numerous shell glands present in oviparous species, unlike viviparous species which had very few shell glands present. The uterine epithelium also differed among species and this may be associated with calcium secretion. The mechanism of calcium secretion needs further study with more specialised techniques.

Differences were noted in the epithelial layer of other regions, specifically the infundibulum and vagina. These differences could not be directly related to differences in parity mode. The possibility that species analysed were at different stages in relation to the time of ovulation should be considered, as it could have profound effects on the appearance of the epithelial layer analysed. It is apparent from the literature that little is known about the oviduct and how it changes during ovulation and the early periods of gestation, a time when vital processes in the oviduct are occurring. Thus, despite several excellent general descriptions available concerning oviduct structure in reptilian species, detailed information concerning oviductal structure, function and control over the important period of ovulation and eggshell formation is lacking.
Chapter Four

Ultrastructure of the uterus in an ovariectomised gecko (Hemidactylus turcicus) after administration of exogenous estradiol

Abstract

Introduction

Materials and Methods
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  Ovariectomy
  Experimental Protocol
  Transmission Electron Microscopy
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Results
  Uterine Epithelium
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Discussion
ABSTRACT

The uterus of an oviparous gecko, *Hemidactylus turcicus*, was analysed after ovariectomised females underwent a period of treatment (up to 14 days) with exogenous estradiol. Analysis focused on the uterine mucosa which is made up of an epithelial layer and an underlying *lamina propria* containing the shell glands. It is these tissues which are thought to be responsible for secretion of the eggshell components and were thus chosen for analysis using transmission electron microscopy. In ovariectomised females, the epithelial layer was low and cuboidal with minimal/no differentiation or secretory activity. Treatment with exogenous estradiol resulted in a significant increase in cell height associated with gradual differentiation of the epithelium. Development of non-ciliated cells included production of secretory granules (low electron density) at the apical cell surface. The shell glands showed less obvious changes over the course of treatment. Shell glands contained two cell types: dark cells with darkly staining nuclei and organelles, and light cells with very indistinct nuclei and organelles, except for prominent rough endoplasmic reticulum and free ribosomes. This study provides results consistent with published light microscopy studies for other reptiles and additionally provides ultrastructural details of reptilian uterine development not previously available.
INTRODUCTION

The reptilian oviduct is derived from the embryonic Müllerian duct (Wake, 1985). Several distinct regions can be observed (Palmer and Guillette, 1988), including a uterus which, in oviparous species, is responsible for secretion of the eggshell (Guillette et al., 1989; Palmer et al., 1993). The uterus itself is made up of several different tissue layers (H. Fox, 1977; Wake, 1985). The innermost layer, the mucosa or endometrium, consists of an epithelial layer lining the lumen of the uterus, with an underlying lamina propria of connective and glandular tissue. Under the mucosa is the muscularis which has both an inner circular and outer longitudinal muscle layer. The outermost tissue layer, the serosa, is continuous with the peritoneum.

The reptilian eggshell has two major components: a calcareous layer and an underlying fibrous layer or shell membrane (M. Packard et al., 1982; M. Packard and DeMarco, 1991). In squamates, the fibrous layer of the eggshell is thought to be secreted by alveolar (shell) glands present in the uterine mucosa, whilst the calcareous component is thought to be secreted by the uterine epithelial cells (Guillette et al., 1989; Palmer et al., 1993).

Limited literature is available concerning the action of steroid hormones in the uterus of reptilian species. It is known that the uterus undergoes changes over the course of a seasonal reproductive cycle (Abrams Motz and Callard, 1991; Perkins and Palmer, 1996) and these changes correlate with seasonal hormone patterns (for example: I. Callard et al., 1978). For instance, during vitellogenesis, estradiol is secreted by the ovary. At the same time, development and hypertrophy of the uterus occurs in preparation for gravidity (Abrams Motz and Callard, 1991; Perkins and Palmer, 1996). Changes which occur during uterine development include an increase in the number of gland cells and an increase in secretory granules in both the epithelial and glandular tissue of the uterus. Similar development and hypertrophy is seen at the light microscope level in non-reproductive or ovariectomised females after administration of exogenous estradiol (La Pointe, 1969; Prasad and Sanyal, 1969; Yaron, 1972; Mead et al., 1981; Abrams Motz and Callard, 1991). That estradiol acts directly on the uterus is implied by the presence of estrogen receptors, as reported in the lizard Podarcis sicula sicula (Paolucci and DiFiore, 1994). In addition, the concentration of estrogen receptors in the uterus of P. s. sicula increases during the period of oviductal growth in the spring (Paolucci et al., 1992).
The general morphology (described using light microscopy) of the reptilian uterus has been documented (for instance, in Gekkonidae: Picariello et al., 1989; Chapters 2 and 3). However, very little information is available concerning the ultrastructural detail of uterine tissue, particularly changes associated with the reproductive cycle or with hormonal manipulation. Therefore, in this study, I analysed uterine tissues (particularly the epithelial and glandular tissue) from the oviparous Mediterranean gecko, Hemidactylus turcicus. Ovariectomised females underwent a period of estradiol treatment, after which the ultrastructure of uterine tissues was analysed using transmission electron microscopy (TEM). I expected that development and hypertrophy of the oviduct would occur after estradiol treatment, as has been reported in other studies (La Pointe, 1969; Prasad and Sanyal, 1969; Yaron, 1972; Mead et al., 1981; Abrams Motz and Callard, 1991). Developmental changes expected included cellular differentiation and hypertrophy in the epithelial layer, an increase in the number and size of endometrial glands and the production of secretory granules in both glandular and epithelial cells. Ultrastructural details associated with estradiol treatment will be discussed.

MATERIALS AND METHODS

Animal Collection and Maintenance

Female geckos (Hemidactylus turcicus, 50-60 mm snout-vent length) in early vitellogenic condition were captured off building walls and windows in Gainesville, Florida, USA, in May, 1996 (n=27). Animals were housed in plastic containers (approximately 35 x 35 x 25 cm) in a temperature controlled room (31-32°C) with ambient photoperiod. Water was provided ad libitum and geckos were fed crickets every 2-3 days.

Ovariectomy

Females were ovariectomised after anaesthesia with AErrane (Isoflurane). A small incision was made just left of the body mid-line (to avoid a blood vessel) on the ventral surface of the abdomen. Both ovaries were located from this incision and removed from the body by cautery. Ovaries were preserved in Bouin's fixative for possible future use. After surgery, the incision was sutured and the wound covered with a small amount of tissue glue. Females recovered quickly
from anaesthesia (usually 5-10 minutes). Females were left for a one month period prior to estradiol treatment to allow recovery from surgery and regression of uterine tissues.

**Experimental Protocol**

At the completion of the recovery period, females were allocated to one of nine treatment groups (n=3 per group). Six of these groups were estradiol-treated groups. Treatment consisted of intraperitoneal injections (50 μl) of estradiol-17β [Sigma, St Louis, USA, 2 μg/ml in buffered Ringer (Guillette, 1982) solution, approximately 25 ng/g body weight] given daily (between 0830 and 1000 am). Groups were dissected after 1, 3, 5, 7, 9 or 14-days of estradiol treatment.

The estradiol dosage used in this experiment was based on a previous study with *H. turcicus* which implanted hormone pellets (intra-peritoneal, 14-day slow release) into ovariectomised females (L.J. Guillette, pers. comm). Treatment with the pellets resulted in oviductal development.

The remaining three groups were termed control-groups. The first of these (vehicle-only control group) received injections of vehicle solution only (buffered Ringer solution) for 14-days prior to dissection. The final two groups received no injections and were dissected at 0 and 14-days (0-day control group and 14-day control group).

Animals were killed by decapitation (swift blow to the head followed by spinal severance) and a blood sample was taken from the vessels at the neck. However, the volume of plasma obtained was insufficient for analysis of plasma estradiol concentration by radioimmunoassay.

Oviductal structure from naturally vitellogenic females has been analysed in a previous study (Chapter 3) and provided a comparison for females from this experiment.

**Transmission Electron Microscopy**

After dissection, one of the two oviducts in each female was removed (left and right oviducts were removed alternatively from different females) and the uterus separated from other regions. Uterine tissue was cut into small pieces.
(approximately 1 mm²) using a sharp razor blade. The tissue was then processed for transmission electron microscopy. Tissues were initially preserved in 2.5% glutaraldehyde in phosphate buffer for 2.5 h, then post-fixed in 1% osmium tetroxide/1.5% potassium ferrocyanide in cacodylate buffer for 1 h. Tissues were then dehydrated down an ethanol series and infiltrated with agar resin. Semi-thin and ultra-thin sections were cut using an Ultracut E (Leica Instruments Pty Ltd, Australia). Semi-thin sections were used in morphometric analysis (see below). Ultra thin sections were viewed on a Philips EM410 microscope (Holland).

**Light Microscopy**

Additionally, uterine tissues from the second oviduct of several individual females (one female from: 9-day and 14-day estradiol treated groups, and 0-day and no-injection control groups; two females from: 3-day estradiol treated group) were processed for light microscopy. Uterine tissues from the remaining experimental females were not available for light microscopy due to their loss in a laboratory accident. Uterine tissues processed for light microscopy were also to be used for immunocytochemistry to analyse the distribution of insulin-like growth factor-I (Chapter 6). However, because of the accident, this could not be undertaken.

Tissues were fixed in Bouin's fixative. They were then washed and dehydrated in ethanol (70%, three x 100%), cleared in xylene, and embedded in paraffin. Sections (6-8 μm) were cut and mounted on glass slides. Slides were treated with a variety of stains (Humason, 1979; Sheehan and Hrapchak, 1980) including: Lillie-Mayer haematoxylin and eosin (H&E, general histology) with an Alcian blue (pH 2.5) counterstain (stains both carboxylated and sulfated acid mucosubstances), Mallory's trichrome (general histology and connective tissue), and periodic-acid-Schiff's reagent (PAS, stains a variety of carbohydrates, including some carbohydrate-protein and carbohydrate-lipid compounds) with a fast green counterstain. Representative slides from each female were subjected to the diastase/saliva test prior to staining with PAS. No differences between slides treated with or without saliva were noted. This eliminates glycogen as a possible staining product.
Morphometrics

Epithelial cell height was estimated by measuring 10 cells per animal from semi-thin sections. Where possible, measurements were taken from cells where both the basal and apical cell surfaces were visible. Results were analysed using Datadesk (New York, USA). A general linear model was used with 'individual females' nested within treatment group. Results are presented as mean ± SE. The coefficient of variation for measurements taken from individual females ranged from 9-28%.

RESULTS

Uterine Epithelium

Light Microscopy

In tissues from the 14-day control group female, the cuboidal epithelial layer was undifferentiated and showed no staining for carbohydrate with either PAS or Alcian blue (Fig. 4.1b). The nuclei were large, and there was relatively little cytoplasm. This pattern was also seen in tissues from the 0-day control group individual (Fig. 4.1a). In the two 3-day estradiol treated females, the epithelium was very similar to the 14-day control and 0-day control group tissue (Fig. 4.1c). Slightly more cytoplasm surrounding the nuclei was noted, as was a fine layer of carbohydrate positive staining on the apical surface (positive with PAS and Alcian blue). In tissue from the 9-day estradiol treated female (Fig. 4.1d), this layer of positive staining was thicker and more distinctive. The female from the 14-day estradiol treatment group (Fig. 4.1e) had considerable carbohydrate staining within the cytoplasm of non-ciliated cells. Ciliated cells could be easily distinguished. The epithelium was pseudo-stratified columnar with elongated nuclei, and resembled that seen in naturally vitellogenic females (Fig. 4.1f).

These observations corresponded well with the morphometric measurements. In comparison to control groups, epithelial cell height increased significantly ($F_\text{d.f.}=8) = 23.0, p \leq 0.0001$) over the period of estradiol treatment (Fig. 4.2).
Figure 4.1. Light micrographs of uterine tissue from ovariectomised geckos, Hemidactylus turcicus. A: 0-day and B: 14-day control females, and C: 3-day, D: 9-day and E: 14-day estradiol treated females; and F: uterine tissue from a naturally vitellogenic Hemidactylus turcicus. Epithelium: e; lumen: l; muscularis: m; uterine glands: g, arrow: layer of positive PAS staining. Scale bars: 100 μm.
Uterine ultrastructure in a gecko

A

m
g
e

B

m
g
e

C

m
g
e

D

m
g
e

E

m
g
e

F

m
g
e
Figure 4.2. Uterine epithelial cell height in individual ovariectomised geckos, *Hemidactylus turcicus*, after up to 14 days of estradiol treatment. Error bars represent standard error.

Epithelial height in the vehicle-only control group was higher than expected when compared with other control groups. Tissue available from semi-thin sections from the vehicle-only control group had few epithelial cells with both apical and basal surfaces visible. This may have distorted the values obtained for the vehicle-only control group.

**Ultrastructural Analysis**

The epithelial layer showed considerable variation among females within the control groups (Fig. 4.3). In certain individuals, it appeared regression of uterine tissues was incomplete (with some cells showing degenerating cytoplasm and organelles). However, certain trends were noted. The epithelial layer was made up of cuboidal-columnar cells with large, circular nuclei. Different nuclei within individual females tended to contain consistent amounts of heterochromatin. However, amounts of heterochromatin varied among females. A prominent nucleolus was often seen. Nuclei were large in relation to the volume of
Figure 4.3. Transmission electron micrographs of uterine epithelium from ovariotomised geckos, *Hemidactylus turcicus*. A: O-day, B: 14-day and C: Vehicle-only control female. Note unusual vesicles (v) present in apical region of epithelial cells in this particular individual. Basement membrane: small black arrow; ciliated cells: large open arrow; connective tissue: c; darkly staining cells: d; lightly staining cells: p; lipid droplets: small white arrow; microvilli: large white arrow; mitochondria: m; uterine lumen: l. Scale bars: 5 μm.
cytoplasm. Golgi apparatus was often seen, but it was not extensive. The apical region of many cells contained small membrane bound vesicles (apparently empty) and small circles and strands of rough endoplasmic reticulum (RER). Mitochondria were numerous and in many cells tended to be large. Very few or no secretory granules were present in epithelial cells. Occasionally, structures consistent with lipid droplets were noted, particularly at the basal region of the cells. Individual epithelial cells often varied in staining intensity in comparison to neighbouring cells (Fig. 4.3a). In cells with a pale staining intensity, organelles were indistinct, except for the numerous RER and free ribosomes.

In general, no differentiation of the apical surface was seen in control females (Figs. 4.3a,b). However, very occasional ciliated cells were noted in some individuals. Cilia on these cells tended to be short, and often seemed to be lying just under the apical surface of the cells (Fig. 4.3a). The cytoplasm of these ciliated cells contained numerous small, fibrous granules (Fig. 4.1b). Some cells had microvilli and/or irregularly shaped cytoplasmic extensions at the apical surface. The glycocalyx was distinguishable, but tended to be low and fine (Fig. 4.3a). Although the apical surface tended to be relatively uniform, in some instances cells (and their contents) appeared to be protruding into the uterine lumen. In one particular female (vehicle-only control group), the cytoplasm was dominated by large vesicles and mitochondria (Fig. 4.3c). The epithelium in this female was not consistent with other individuals.

Females from estradiol treated groups 1-day (Fig. 4.4a) and 3-day (Fig. 4.4b) showed few differences from females not receiving estradiol treatment. That is, their epithelial cells showed a tendency for large rounded nuclei, few, if any secretory granules, and only the occasional ciliated cell. There was relatively little cytoplasm in the epithelial cells with comparatively small numbers of organelles.

In females from the 5-day (Fig. 4.4c), 7-day (Fig. 4.4d) and 9-day (Figs. 4.5a,b) estradiol treated groups, some differences began to be noted. As with groups discussed above, there was considerable variation among females in the different treatment groups. Definite differences from controls were not obvious until females from the 9-day treatment group were examined.

A trend could be seen in the epithelium: cells in control group females were cuboidal, whereas after 14 days of estradiol treatment, the epithelium was pseudo-stratified columnar (Fig. 4.5a). The nuclei reflected this trend, with circular nuclei
Uterine ultrastructure in a gecko

Figure 4.4. Transmission electron micrographs of uterine epithelium from ovariectomised geckos, *Hemidactylus turcicus*. A: 1-day, B: 3-day, C: 5-day and D: 7-day estradiol treated females. Darkly staining cells: d; Golgi apparatus: black arrow; lightly staining cells: p; lumen: l; nuclei: n; secretory granules: s. Scale bars: 5 μm.
Figure 4.5. Transmission electron micrographs of uterine epithelium from ovariectomised geckos, *Hemidactylus turcicus*. A-B: 9-day and C-D: 14-day estradiol treated females. Cilia: black arrow; cytoplasmic extensions: open arrow; lumen: l; microvilli: white arrow; secretory granules: s. Scale bars: A-B: 5 μm, C-D: 1 μm.
in control groups and elongated nuclei in 14-day estradiol treated females. More ciliated cells (Fig. 4.5b) were noted, often with microvilli present amongst the cilia. Some cells appeared to be in the process of developing cilia, as cilia were no taller than adjacent microvilli. Others cells had unusually shaped cytoplasmic extensions extending from the apical surface. In some cells, secretory granules (low electron density) were noted at the apical surface, especially after 9-days of estradiol treatment. These apparently filled or replaced small vesicles and pieces of RER noted in earlier stages of the experiment. Other cells, however, had the appearance of those previously described for females not receiving any estradiol. The variability in cell types present gave the apical surface an irregular appearance. Individual cells varied considerably in their staining intensities (Figs. 4.4b,d).

At the completion of the experiment, females receiving estradiol for 14-days had a developed epithelium, resembling that seen in naturally vitellogenic females (Chapter 3), with both ciliated and non-ciliated cells (Figs. 4.5c,d). The epithelium was pseudo-stratified columnar with elongated nuclei. Ciliated cells had numerous mitochondria, predominantly above the nucleus (Fig. 4.5c). Microvilli were also present amongst the cilia. The glycocalyx was distinctive on the epithelial cell surface, especially amongst the cilia and microvilli. Non-ciliated cells had large secretory granules of low electron density at the apical surface (Fig. 4.5d). These granules often appeared to be coalescing. Some had been released, or displayed a morphology suggestive of preparation for release, into the uterine lumen. Small membrane-bound blebs containing secretory material were sometimes seen protruding into the lumen. In some non-ciliated cells, long, cytoplasmic extensions into the lumen were observed (Fig. 4.5c). Non-ciliated cells had very few, if any, microvilli on their apical surface (Fig. 4.5d).

In all epithelial cells, strands of rough endoplasmic reticulum, as well as many free ribosomes, were found throughout the cytoplasm. Golgi apparatus were numerous as were large mitochondria. Lipid droplets were occasionally seen at the basal regions of the cells.

**Uterine Glands**

Two types of gland cells were distinguished (Fig. 4.6). The first type of cell (light cells) were consistently of a low electron and staining density. The nucleus and organelles were indistinct. However, free ribosomes and rough endoplasmic
Figure 4.6. Light micrograph of uterine tissue from an ovariectomised gecko, *Hemidactylus turcicus*, after 7-days of estradiol treatment. Note: dark staining cells (black arrow) and light staining cells (white arrow). Epithelium: e. Scale bar: 50 μm.

reticulum were easily distinguishable and seen throughout the cytoplasm. In the second cell type (dark cells), the cell nuclei and cytoplasm were considerably more dense and organelles were very distinct.

Light Microscopy

In the 0-day and 14-day control group females, and the 3-day estradiol treated female, glands were surrounded by large amounts of connective tissue (Figs. 4.1a,b,c). The cells were small and cuboidal with very little cytoplasm visible. In tissue from 9-day (Fig. 4.1d) and 14-day (Fig. 4.1e) estradiol treated females, and in naturally vitellogenic females (Fig. 4.1f), gland cells were more columnar with nuclei situated basally. Considerably more cytoplasm was visible. Many more glands were present in the mucosa and very little connective tissue was present.
in comparison to previous groups. Gland cells did not stain with either of the carbohydrate stains.

Ultrastructural Analysis

In females from the control groups, glands tended to be surrounded by large amounts of connective tissue (Figs. 4.7a,b). Nuclei were large and contained variable amounts of heterochromatin. Relatively small amounts of cytoplasm were present. Considerable interdigitation of dark staining cell membranes between glandular cells could be seen. Occasional microvilli were found on the apical surface of glandular cells lining the lumen. Usually, no material was noted in the lumen of glands. The glands were made up predominantly of light cells with obvious rough endoplasmic reticulum and ribosomes. However, some dark cells were present. In these cells, distinct areas of Golgi apparatus were apparent, mitochondria were common and easily distinguished and various vesicles (sometimes large) and lysosomes were also visible. Numbers of secretory granules varied between individual cells. In some, few granules were visible in the plane of section. In other, granules were numerous and were seen to be in the process of formation. What appeared to be lipid droplets were also seen in the cytoplasm.

Ultrastructural changes in gland cells were not as distinct as differences noted in the epithelial cells. Few differences were noted in females from 1-day, 3-day and 5-day estradiol treated groups in comparison to females not receiving estradiol treatment.

However, females in the 7-day (Fig. 4.7d) and 9-day (Fig. 4.7c) estradiol treated groups did exhibit some differences. Dark cells tended to make up glands that were closer to the epithelium. Light cells were in glands nearer to the muscle layer. This trend, however, was not consistent in all females and many glands were still made up of a mixture of light and dark cells. There was also a reduction in the amount of connective tissue which surrounded the glands. Numerous secretory granules were usually seen in individual gland cells, as were occasional lipid droplets. Granules in the process of formation were common, as were various vesicles.

In females from the 14-day estradiol treated group, glands tightly filled the mucosa with little connective tissue. Aggregations of heterochromatin were
**Figure 4.7.** Transmission electron micrographs of uterine glands in ovariectomised geckos, *Hemidactylus turcicus*. A: 0-day control, B: vehicle-only control, C: 3-day and D: 7-day estradiol treated females. Connective tissue: c; darkly staining cells: d; Golgi apparatus: black arrow; interdigitation of gland cell membranes: large white arrow; lightly staining cells: p; lipid droplets: small white arrow; lumen: l; nuclei: n; secretory granules: s. Scale bars: 5 µm.
Present in the nuclei. Individual gland cells consistently contained numerous secretory granules (Figs. 4.8b,c). Both light and dark cells could be seen, but differences between the two cell types were not as distinct as the staining intensity between cell types was not as strong. Mitochondria were common and tended to be elongate and relatively small. Other vesicles containing various amounts of granular material were common. Interdigititation between cell membranes was often seen. Extensive areas of RER and Golgi apparatus were present.

Many cells from glands in the 14-day estradiol treated females appeared unhealthy (Fig. 4.8b). In places, the cytoplasm was breaking apart and vesicles also seemed to be disintegrating. This may be degradation due to tissue processing or may represent dying cells. Similar observations were made for naturally vitellogenic females, except differences between light and dark gland cells were not noted (Chapter 3).

**DISCUSSION**

Administration of exogenous estradiol to ovariectomised, oviparous geckos (*Hemidactylus turcicus*) resulted in changes in uterine tissue including an increase in epithelial cell height, increased secretory activity in the epithelial layer, and a reduction in the amounts of connective tissue surrounding gland cells. Similar results concerning the uterus and other regions of the oviduct have been reported for other reptilian species at the light microscope level (*Klauberina riversiana*, La Pointe, 1969; *Hemidactylus flaviviridis*, Prasad and Sanyal, 1969; *Sceloporus cyanogenys*, I. Callard et al., 1972b; *Xantusia vigilis*, Yaron, 1972; *Cnemidophorus inornatus* and *C. neomexicanus*, Christainsen, 1973; *Thamnophis elegans*, Mead et al., 1981; *Chrysemys picta*, Abrams Motz and Callard, 1991). My study shows that these generalised changes are associated with more detailed ultrastructural changes. The increase in secretory activity in the uterus of *H. turcicus*, for instance, was a result of the development of low electron dense secretory granules in the apical region of non-ciliated cells.

Similar developmental effects of exogenous estradiol are noted in other vertebrate groups. In birds and mammals, unlike reptiles, a substantial body of research is available concerning ultrastructural detail of the reproductive tract and the influence of estradiol. For instance, administration of estradiol to ovariectomised cats restored the Fallopian tube to a fully differentiated state
Figure 4.8. Transmission electron micrographs of uterine glands from ovariectomised geckos, *Hemidactylus turcicus*. A: 9-day and B-C: 14-day estradiol treated females. Darkly staining cells: d; Golgi apparatus: black arrow; lightly staining cells: p; lipid droplet: white arrow; lumen: l; nuclei: n; secretory granules: s. Scale bars: A-B: 5 μm, C: 1 μm.
(Verhage and Brenner, 1975; Bareither and Verhage, 1981), a process which included ciliogenesis (a multiphase process discussed in detail in Verhage and Brenner, 1975). Additional ultrastructural changes in the Fallopian tube of the ovariecotomised cat include an increased amount of polyribosomes, mitochondria and Golgi apparatus over the course of estradiol treatment (Verhage and Brenner, 1975). Differentiation of secretory cells included development of basal rough endoplasmic reticulum and a large Golgi region. Secretory granules were noted after three days of estradiol treatment (Bareither and Verhage, 1981).

Ultrastructural changes noted by Verhage and Brenner (1975) included the conversion of chromatin from a condensed to a dispersed state. A consistent change of chromatin state was not noted in *H. turcicus* during this study. This may be due to individual variation among the different females.

The process of ciliogenesis and secretory cell differentiation was also reported to be estradiol dependent in the chick oviduct (Anderson and Hein, 1976), cat uterus (Bareither and Verhage, 1980) and the Fallopian tube of the baboon (Verhage *et al.*, 1990), pig-tailed monkey (Odor *et al.*, 1980) and the newborn golden hamster (Abe and Oikawa, 1993a).

Prior to estradiol treatment, female geckos were ovariecotomised to remove the natural source of estradiol and allow the uterine tissue to regress. Mead *et al.* (1981) noted that ovariecotomy significantly reduced the height and secretory activity of both luminal and glandular epithelium, although numerous cells were still ciliated. In this study also, a few ciliated cells were noted in ovariecotomised females. This may indicate that the uterine tissue had not fully regressed in the time period between ovariecotomy and the beginning of estradiol treatment. It may also be that a remnant population of ciliated cells remained in the regressed uterus for basic functions such as movement of mucus and cellular debris.

The general structure of the uterus (and other regions of the reptilian oviduct) has been described (Palmer and Guillette, 1988; Picariello *et al.*, 1989; Perkins and Palmer, 1996). However, very little ultrastructural detail is available (Aitken and Solomon, 1976; Palmer and Guillette, 1992; Chapters 2 and 3). I have previously (Chapter 3) described the uterus of *H. turcicus* during the later stages of vitellogenesis. At this stage, the uterus shared similarities with the uterus in ovariecotomised females after estradiol treatment. This included numerous secretory granules within glandular cells, some of which appear to be in the
process of formation. In the epithelial layer, non-ciliated cells contained secretory granules of low electron density in the apical region of the cell. Organelles present within the cells were the same.

Ultrastructural information provided by Aitken and Solomon (1976) was based on two animals. They described two glandular regions within the oviduct of the green turtle *Chelonia mydas*. The more caudal of these regions was the "region of shell formation" or uterus. As with *H. turcicus*, glandular cells contained variable numbers of osmiophilic secretory granules. Golgi apparatus was prominent and complex. Unlike *H. turcicus* in this study, however, gland cell boundaries were described as uncomplicated with few interdigitations. In the alligator (*Alligator mississippiensis*), the uterus was divided into anterior and posterior regions (Palmer and Guillette, 1992). The anterior uterus produced the shell membranes, and the glands in this region contained numerous electron-dense secretory granules. In the posterior uterus, which secretes the calcareous component of the eggshell, gland cells did not contain secretory granules (Palmer and Guillette, 1992).

Ultrastructural information available in the literature for reptiles does not discuss differences of staining intensity in the glandular (light and dark cells) or epithelial cells. In ovariectomised cats, differences in staining intensity of epithelial cells was related to cell type (Verhage and Brenner, 1975). Ciliated cells were less osmiophilic (lower staining intensity) than non-ciliated cells. This may well be the case in *H. turcicus*. Lighter staining cells in the epithelium may be those which will ultimately differentiate into ciliated cells. Differences in staining intensity were most distinct in cells of the shell glands. However, no ultrastructural information is available for *H. turcicus* in the postparturition/early vitellogenic reproductive stages. Therefore, it is possible that differences in gland cell staining intensity may represent an artefact of hormone manipulation. Differences may also represent differences in the function or activity level of different cells. More research is needed to distinguish between these possibilities.

At the completion of estradiol treatment, the uterus of *H. turcicus* resembled the uterus of naturally vitellogenic females (Chapter 3). However, although estradiol is known to be the primary hormone associated with oviductal development, it is highly unlikely to act in isolation. In *Cnemidophorus inornatus* and *C. neomexicanus*, oviductal hypertrophy could be induced by estriol, estrone, or
estradiol (Christainsen, 1973). Limited development was also noted with administration of testosterone and testosterone propionate. In non-reproductive *Lacerta sicula*, administration of pregnant mare serum (PMS) stimulated the ovaries, as well as causing hypertrophy of the oviduct (Botte and Basile, 1974). In ovariectomised females, PMS did not induce morphological changes. However, increased levels of DNA and protein content, and phosphatase activity, were noted (Botte and Basile, 1974). This suggests gonadotropins could act directly, or at least independently of ovarian steroids, on the oviduct.

Estrogen receptors have been noted in the oviduct of reptilian species and their concentration changes over a reproductive cycle (for instance: Paolucci et al., 1992; Paolucci and DiFiore, 1994; Giannoukos and Callard, 1996). In *Podarcis s. sicula*, estradiol receptor concentration increased during the period of oviduct growth (vitellogenesis) in the spring (Paolucci et al., 1992; Paolucci and DiFiore, 1994). Receptor levels were higher in the cytosol in quiescent as compared to vitellogenic oviducts, but during growth there was an increase in nuclear sites. In non-reproductive, ovariectomised females, estradiol treatment increased estrogen receptor number and caused a shift of receptors from the cytosol into the nuclear component (Paolucci et al., 1992; Paolucci and DiFiore, 1994). In the turtle *Chrysemys picta*, ovariectomy significantly reduced estrogen receptor levels in the oviduct (Giannoukos and Callard, 1996). However, administration of estradiol did not restore estrogen receptor levels. This is an unusual result when considering the effects of estradiol on oviductal development. If estrogen receptor number usually increases during vitellogenesis, a similar effect would be expected in ovariectomised females after estradiol treatment.

The mechanism by which the reptilian eggshell, produced by the uterus, is secreted is not fully understood. The fibrous component of the eggshell is secreted by the alveolar glands found in the uterus (Guillette et al., 1989; Palmer et al., 1993). Material secreted by the endometrial glands coalesces into a fibre as it is extruded from the gland opening (Palmer and Guillette, 1992; Palmer et al., 1993). The fibre is then wrapped around the egg. Geckos produce up to two large, yolky eggs. In *Hoplodactylus maculatus* (viviparous), the presence of these eggs in the uterus after ovulation caused tissues to be highly stretched, leaving only a very fine epithelial layer covering the muscularis (Chapter 2). The connective tissue of the mucosa was hardly visible, if at all, and the few glands which were present during vitellogenesis were absent during pregnancy. If similar stretching is seen in oviparous gecko species, including *H. turcicus*, it suggests a mechanism
by which the shell membrane is secreted. As the egg enters the uterus it stretches the epithelial and glandular cells of the mucosa. This may trigger or force the gland cells to release their secretory contents. Or, individual glandular cells (or a subset of cells) may themselves be budded off, forming the shell membrane. These possibilities are supported by certain features. Both the epithelium and gland cells of H. turcicus were bounded by a thick basement membrane suggesting that stretching is possible. In H. turcicus, at the completion of estradiol treatment and in vitellogenic females, many of the gland cells appeared to be dying. These may be cells which will be budded off and form part of the eggshell fibres. In other reptilian species, this extreme stretching of the uterus is not seen (Guillette et al., 1989) and glands remain intact.

The process of eggshell formation has been described in birds (Tullett, 1987). Certain details, however, appear to be sketchy. The calcium needed for the eggshell for instance, is secreted in the tubular shell gland and shell gland pouch regions. In several instances, it is the gland cells of these regions which are reported to be responsible for secretion of the calcium component (Johnston et al., 1963; Breen and DeBruyn, 1969; Wyburn et al., 1973; Tullett, 1987). In another report, the epithelial layer of these regions is thought to be responsible for secretion of the calcareous component (Solomon et al., 1975). Solomon et al. (1975) used potassium pyroantimonate to localise/precipitate calcium ions. Deposits were seen in epithelial cells of both the tubular and pouch regions of the shell gland, but not in the isthmus, and no deposits were seen in the tubular gland cells of either the shell gland or isthmus. In reptiles also, the source of calcium is not known for sure, although some evidence suggests the epithelial layer is responsible. For instance, in Crotaphytus collaris but not Eumeces obsoletus, secretory cells in the uterus stain positively for calcium during gravidity (Guillette et al., 1989). Further specialised techniques will be required to visualise the source of calcium in H. turcicus and elucidate the hormonal mechanisms controlling its sequestration and secretion.

In summary, treatment of ovariectomised female geckos caused hypertrophy and differentiation of the uterine tissue. Ultrastructural changes associated with an increase in epithelial cell height and secretory activity have been discussed. Unusual cell types were noted, particularly in the shell glands. The reason for these differences is not known, although it may reflect differences in the function of cells. The results from this study are consistent with other reports in reptilian
species, as well as mammals and birds, but also provide further ultrastructural information not previously available for reptiles.
Chapter Five

Unexpected results in a study analysing the effects of progesterone on oviductal structure in estrogen-primed New Zealand common geckos (*Hoplodactylus maculatus*)

Abstract

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Discussion
Oviductal tissues from New Zealand’s common gecko, *Hoplodactylus maculatus*, were analysed using light and transmission electron microscopy in ovariectomised females following treatment with either estradiol (four or six weeks), estradiol (four weeks) followed by progesterone (two weeks), or vehicle solution only. The experiment was undertaken to determine the effects of progesterone on the oviduct in estrogen-primed female geckos. After a two month recovery period following ovariectomy, hormone was administered by intraperitoneal injections every four days for the appropriate treatment period. Unexpectedly, no differences were noted in plasma estradiol concentrations or oviductal ultrastructure among the four different treatment groups. Oviductal tissue resembled that observed in wild, vitellogenic females and no sign of regression was noted in the vehicle-only treatment group. Potential reasons for the high plasma estradiol concentrations in all treatment groups include a reduced clearance rate of estradiol from the body, secretion of estradiol by the adrenal gland, or secretion of androgens by the adrenal gland followed by aromatisation to estradiol by the brain or the oviduct itself. No additional changes were noted in the oviduct of progesterone-treated females in comparison to female treated with estradiol only. Although the lack of effect by progesterone is consistent with certain other studies, it is possible that results are confounded by the unexpected plasma estradiol concentrations. Further studies are needed to elucidate the potential sources of plasma estradiol following ovariectomy in this gecko before studies concerning the additional effects of progesterone can be undertaken.
INTRODUCTION

In reptilian species, estradiol, which is secreted by the ovary during vitellogenesis, influences the development of the oviduct. Oviductal development can be induced by administering exogenous estradiol to ovariectomised females (Prasad and Sanyal, 1969; I. Callard et al., 1972b; Yaron, 1972; Christainsen, 1973; Botte et al., 1974; Paolucci and DiFiore, 1994; Chapter 4). The effects of estradiol treatment include hypertrophy and differentiation of the oviductal epithelium, and recruitment of shell glands in the uterine region.

Another hormone which influences oviductal action is progesterone. Plasma progesterone concentrations in female reptiles show a seasonal pattern, with the highest concentrations often occurring around the time of ovulation and through gravidity (Chan et al., 1973; Veith, 1974; Licht, 1982; Xavier, 1982; Dauphin-Villemant et al., 1990). The presence of progesterone over this period is attributed, in part, to the presence of a functional corpus luteum. In oviparous species, the decline in plasma progesterone concentrations is correlated with the regression of the corpora lutea prior to or soon after ovulation. In viviparous species, however, the seasonal changes in plasma progesterone concentration and the timing of luteal regression varies considerably among species (R. Jones and Baxter, 1991; I. Callard et al., 1992). The adrenal gland is also known to secrete progesterone (Dauphin-Villemant and Xavier, 1985).

Progesterone has been implicated in various aspects of oviductal control in reptiles, although specific functions are not understood. That progesterone does act directly on the oviduct, however, is implied by the presence of progesterone receptors (Chrysemys picta, Ho and Callard, 1984; Reese and Callard, 1989; Trachemys scripta, Selcer and Leavitt, 1991; Podarcis s. sicula, Paolucci and DiFiore, 1994). The actions of progesterone include modification of contractility in the turtle oviduct (I. Callard and Hirsch, 1976; Abrams Motz and Callard, 1988), induction of avidin (egg-white protein) production in Podarcis s. sicula (Botte et al., 1974; Botte and Granata, 1977), and increasing the vascularity of reproductive tissues in various lizard species (Veith, 1974; Masson and Guillette, 1987).

Progesterone is also implicated in aspects of oviductal control in mammalian species. Chronic administration of progesterone to estrogen-primed cats results
in cell atrophy, de-differentiation and apoptosis in epithelial cells of the Fallopian tube (Bareither and Verhage, 1981). A short-term response to progesterone is seen in the cat endometrium. An increase in the size of secretory granules along with decreased electron density occurs after only 5-15 minutes of progesterone infusion in estrogen-primed, ovariectomised cats (Bareither and Verhage, 1980). In the pig, progesterone causes the uterus to synthesise and secrete proteins needed to maintain the embryo (Roberts and Bazer, 1988). In the rabbit, release of secretory granules occurs after coitus or administration of progesterone in the estrogen-primed female (Greenwald, 1969).

Only a few studies have looked at the histological structure of the reptilian reproductive tract after estrogen and progesterone administration. Mead et al. (1981) administered exogenous estradiol to ovariectomised viviparous snakes (Thamnophis elegans) which caused partial restoration of epithelial cell height and glandular activity in the uterine region. Administration of progesterone had no stimulatory effects on uterine histology. Simultaneous administration of estradiol and progesterone did not induce any further stimulatory effects than administering estradiol alone (Mead et al., 1981). Similar results were seen in the gecko Hemidactylus flaviviridis (Prasad and Sanyal, 1969). However, in the lizard Xantusia vigilis (Yaron, 1972), differentiation and restoration of normal oviductal structure were obtained when both estradiol and progesterone were administered concomitantly. These studies suggest that, in contrast to mammalian species, progesterone has little or no stimulatory effect on the reptilian oviduct.

However, in studies which have administered exogenous progesterone to reptiles, progesterone has been administered alone or in combination with estradiol (see above; Prasad and Sanyal, 1969; Yaron, 1972; Mead et al., 1981; Paolucci and DiFiore, 1994). Very few studies have considered the effect of progesterone on estrogen-primed reptiles, a treatment which would more closely follow the hormone pattern which is expected during a natural reproductive cycle in many species (estradiol increasing during vitellogenesis, progesterone high around ovulation and/or during gravidity; I. Callard et al., 1978; S. Jones and Swain, 1996; S. Jones et al., 1997). Giannoukos and Callard (1996) did consider changes in receptor number in progesterone treated, estrogen-primed turtles. They found that progesterone receptors increased after ovariectomy, were unaffected by estradiol treatment and decreased in estrogen-primed females treated with progesterone (Giannoukos and Callard, 1996).
What is obvious from the literature is that the functions of progesterone in the reptilian oviduct are not well understood. Functions will undoubtedly differ depending on the stage of the reproductive cycle. In regard to the oviduct as a whole, the actions of progesterone may well differ among the different regions in relation to the function of that region.

The aims of this experiment were to: 1. analyse the effects of exogenous estradiol on the oviduct in ovariecомised New Zealand common geckos (Hoplodactylus maculatus) and 2. to analyse the effects of progesterone on the structure and ultrastructure of the oviduct in estrogen-primed females. I chose H. maculatus for this study as detailed seasonal ultrastructural information concerning the oviduct was already available (Chapter 2). Progesterone was administered to estrogen-primed, ovariecомised females in an attempt to more closely mimic the hormone pattern expected around the time of ovulation. In H. maculatus, progesterone concentrations are low during early vitellogenesis, then peak in pre-ovulatory/post-ovulatory females (A. Cree, J. Girling, J. Rock, unpubl. obs.). I expected that initial ovariecомy, which removes the major source of estradiol, would cause regression of all regions in the oviduct. Administration of exogenous estradiol was expected to cause development of the oviduct with increased differentiation and secretory activity of the epithelial layer. Hypothesised actions of progesterone included: increased vascularity of the uterus (associated with potential formation of a placenta) and secretory activity associated with movement of the follicle down the oviduct. Differences in oviductal structure between vitellogenic and pregnant females suggest possible changes in secretory activity, and vascularity is particularly distinct in pregnant females (Chapter 2). None of these expected results were seen in this study. Possible reasons for this are discussed.

METHODS

Animal Capture and Maintenance

Early-mid vitellogenic geckos (n=20) were collected near the Hyde turn-off from the Macraes Flat-Middlemarch Road in Central Otago, New Zealand in March, 1997. They were captured from under loose rock slabs on schist outcrops. Mature females collected ranged from 66-75 mm snout-vent length.
Geckos were maintained in a constant temperature room (15°C ± 2°C) with a 12:12 h light: dark regime. A heat lamp was provided for six hours daily (gives air temperature approximately 20-25°C). Animals (four per container) were kept in plastic containers (40 x 60 x 20 cm) with cover available and provided with water and fruit *ad libitum*. House flies were given approximately once a week.

**Ovariectomy**

After anaesthesia with halothane (Fluothane, Zeneca Ltd, United Kingdom), females were ovariectomised. A small incision was made just left of the body mid-line (to avoid a blood vessel) on the ventral surface of the abdomen. Both ovariectomised females were treated with halothane. Ovaries were preserved in Bouin’s fixative or 2.5% glutaraldehyde for possible future use. After surgery, the incision was sutured and the wound covered with a small amount of tissue glue (collodion flexible). Females recovered quickly from anaesthesia (usually 5-10 minutes). Following surgery, geckos were left for a 2 mo period prior to experimental treatment to allow recovery from surgery and regression of oviductal tissues. Based on a previous experiment in which *Hemidactylus turcicus* (Chapter 4) showed oviductal regression after a 1 mo recovery period, 2 mo was expected to provide ample time for oviductal regression in the more cool adapted *H. maculatus*.

**Initial Trial**

Hormone dosages used in the main experiment were based on an initial trial performed using females from a laboratory colony. Trial animals were ovariectomised, left to recover for a period of 1 mo, and then given hormone injections (ever four days). Three estradiol injections were given, followed by two progesterone injections. Repeated blood samples were taken (24 h after the second estradiol injection, 72 h after the third estradiol injection, and 24 h after the second progesterone injection) from the palatal sinus using a heparinised syringe and 25 g needle. Blood samples were only taken from three of the five geckos at each sampling period to minimise samples per animal. The trial geckos were killed (by an overdose of anaesthetic and severance of the spinal column) and dissected 72 h after the second progesterone injection and a blood sample was collected (from the back of the neck). The plasma was analysed using radioimmunoassay (RIA, see below) to determine plasma estradiol and progesterone concentrations.
Plasma estradiol concentrations measured in trial animals 24 h after the second estradiol injection and 72 h after the third estradiol injection were $2695 \pm 1409$ pg/ml and $2817 \pm 1353$ pg/ml respectively. This can be compared with plasma estradiol concentration measured in wild, vitellogenic females in April, 1993 ($41 \pm 29$ pg/ml, Girling, 1993). Estradiol dosage (300 ng per injection, 75 ng per day in a 12 g lizard) was based on concentrations injected during a previous experiment with an oviparous gecko, *Hemidactylus turcicus* (Chapter 4) which effectively induced oviductal hypertrophy. The estradiol dose of 300 ng per injection was also used in the main experiment.

I was aiming to achieve a plasma progesterone concentration of 10 ng/ml in progesterone treated females in this experiment (the same as the mean concentration in wild females around the time of ovulation, A. Cree, J Girling and J. Rock, unpubl. obs.). The initial dosage used in the trials was based on a study by Giannoukos and Callard (1996) who administered 5 µg progesterone/g/day to achieve a plasma concentration of around 70 ng/ml (pharmacological). This information was extrapolated; to get a plasma concentration of around 10 ng/ml, 0.71 µg/g/day would be needed (9 µg per day in a 12 g lizard). As injections were to be given every four days, an injection containing 36 µg progesterone was used. Plasma progesterone concentrations measured in trial animals 24 h and 72 h after the second progesterone injection were $6.1 \pm 3.4$ ng/ml and $2.8 \pm 0.7$ ng/ml (mean ± SE) respectively. Based on these results, I decided to use 72 µg progesterone per injection in the main experiment.

**Experimental Design**

At the completion of the recovery period, females were separated into four treatment groups:

1. Females received injections of vehicle only for a six week period.
2. Females received estradiol injections for four weeks.
3. Females received estradiol injections for six weeks.
4. Females received estradiol injections for four weeks, followed by progesterone injections for two weeks.

Two estradiol-treated groups (2 and 3) were required to check that any changes seen after progesterone treatment (group 4) were due to the progesterone and not to the lack of estradiol. Injections were given every four days during the
experimental period. Hormone injections consisted of 300 ng estradiol or 72 µg progesterone in 50 µl vehicle solution (0.7% saline).

One female from each treatment group was maintained in each of the five containers (four animals per container). Females were identified by an individual toeclip, as well as a number drawn on their dorsal surface with a silver pen. Females were killed 24 h after their final injection by an overdose of anaesthesia and severance of the spinal column. A blood sample was collected from the back of the neck using heparinised capillary tubes. [A blood sample was only taken from each animal at dissection. No blood was collected prior to ovariectomy to minimise manipulation and handling of experimental animals. Additionally, the small body size of the lizards means multiple blood samples are difficult to obtain. Previous studies show ovariectomy reduces plasma estradiol concentrations (Mead et al., 1981).] Blood was centrifuged and the plasma removed and frozen at -80°C until analysis for plasma estradiol and progesterone concentrations. Prior to immersion in fixative, the wet weight of both oviducts was obtained and a mean oviductal weight calculated for each female. Mean oviductal wet weight (as a percentage of total body weight) for each treatment group was analysed using a general linear model (Datadesk, New York, USA). One oviduct was fixed for light microscopy, the other was removed and used for transmission electron microscopy (TEM). At the time of dissection, no ovarian tissue remained in the body cavity.

Radioimmunoassay for Plasma Hormones

Thawed plasma was extracted once with freshly redistilled dichloromethane (for estradiol) or hexane (for progesterone) and reconstituted in phosphate buffer for use in steroid RIAs. Recovery in spiked plasma using this procedure was 89.7±1.5% (mean ± SE; n=7) for tritiated estradiol and 76.5±1.2% (n=6) for tritiated progesterone. Plasma steroid concentrations determined in the assay have been corrected for extraction dilution factors and percentage extraction recovery.

Plasma samples were analysed in duplicate using estradiol and progesterone radioimmunoassays validated in Dr. A. Cree's laboratory for plasma from H. maculatus. Tritiated labels were obtained from Amersham (UK) and the antibodies (estriadiol: E26-47, progesterone: P11-192) from Endocrine Sciences, California. Extracted plasma was incubated overnight at 4°C with antibody (estriadiol 1: 2394, progesterone 1: 3020) and label (estriadiol 1:13,400; progesterone
Estradiol and progesterone effects

1:20,000) in phosphate buffer. Bound and unbound phases were then separated by addition of dextran-charcoal followed by centrifugation (15 min, 2000g). The supernatant was counted and spline-fitting of the standard curve performed using a liquid scintillation counter (Packard Instruments Company, Meriden). Plasma concentrations were analysed using a one-way analysis of variance (Datadesk, New York, USA). Samples with non-detectable plasma concentrations were given the minimum detectable value during statistical analysis.

Estradiol Assay

Plasma samples from the main experiment were analysed in two assays with a total binding of 43.6% and 31.1%, and non-specific binding of 2.9% and 2.7% respectively. The minimum detectable concentration (7.8 pg/ml) of the estradiol assay was calculated using 12 zero tubes in a single assay (A. Cree, pers. comm.). The mean zero (no hormone present) value on the standard curve, minus two standard deviations, was determined to be the minimum detectable concentration. After accounting for extraction dilutions and percentage extraction recovery, the minimum detectable concentration in plasma was 24.6 pg/ml.

Intraassay variation was calculated using three quality control with binding levels of 20, 50, and 80 %. Ten tubes of each control were analysed and yielded a mean coefficient of variation of 5.0 % (Girling, 1993). Interassay variation is calculated using the same quality controls as above, but over several assays (10.7 % calculated over 5 assays, A. Cree, pers. comm.). The mean percentage recovery in the assay of gecko plasma extracts spiked with estradiol was 86.7 ± 1.7 % (mean ± SE, n=4, Girling, 1993). Plasma samples showed parallelism to the standard curve when serially diluted.

Progesterone Assay

Plasma samples from the main experiment were analysed in a single assay with total binding of 26.1 % and non-specific binding of 7.0 %. The minimum detectable concentration, calculated as for estradiol, is 0.19 ng/ml on the standard curve (A. Cree, pers. comm.). After accounting for extraction dilutions and percentage extraction recovery, the minimum detectable concentration in plasma was 0.42 ng/ml.
Intraassay variation was calculated using three quality control with binding levels of 20, 50, and 80%. Ten tubes of each control were analysed and yielded a mean coefficient of variation of 12.2% (Girling, 1993). Interassay variation was calculated over 3 assays to be 10.1% (A. Cree and C. Tyrrell, pers. comm). The mean percentage recovery in gecko plasma extracts spiked with progesterone was 88.3 ± 3.2% (mean ± SE, n=5, A. Cree, pers. comm.). Plasma samples showed parallelism to the standard curve when serially diluted (A. Cree, pers. comm).

Light Microscopy

Oviductal tissues for light microscopy were fixed in Bouin's fixative. They were then washed and dehydrated in ethanol (70%, three x 100%), cleared in xylene, and embedded in paraffin. Serial sections (8 μm) of the entire oviduct were cut and mounted on glass slides and representative slides from the infundibulum, uterine tube, and uterus were treated with a variety of stains (Humason, 1979; Sheehan and Hrapchak, 1980) including Lillie-Mayer haematoxylin and eosin (H&E, general histology) with an Alcian blue (pH 2.5) counterstain (stains both carboxylated and sulfated acid mucosubstances), Mallory's trichrome (general histology and connective tissue), and periodic-acid-Schiff's reagent (PAS, stains a variety of carbohydrates, including some carbohydrate-protein and carbohydrate-lipid compounds) with a fast green counterstain. Representative slides from each treatment group underwent the saliva (diastase) test prior to PAS staining (Humason, 1979). No differences were noted between slides treated with or without the test, therefore eliminating glycogen as the staining product.

Transmission Electron Microscopy

Tissues (infundibulum, uterine tube, uterus) from two females per group were processed for TEM. The oviducts were initially preserved in 2.5% glutaraldehyde in phosphate buffer for 2.5 h, then post-fixed in 1% osmium tetroxide/1.5% potassium ferrocyanide in cacodylate buffer for 1 h. Tissues were then dehydrated down an ethanol series and infiltrated with agar resin. Semi-thin and ultra-thin sections were cut using an Ultracut E (Leica Instruments Pty Ltd, Australia). Ultra thin sections were viewed on a Philips EM410 microscope (Holland).
Morphometrics and Statistics

Light micrographs were taken of three of the five oviductal regions (infundibulum, uterine tube, and uterus) from each female. Epithelial cell height was estimated from the micrographs. For each region from every female, 10 cells were measured using callipers (the coefficient of variation for measurements taken from each female ranged from 7.5-31.7%). Results were analysed using Datadesk (New York, USA). A general linear model was used with 'individual females' nested within treatment group. Results are presented as mean ± SE.

RESULTS

Plasma Hormone Levels

Surprisingly, mean plasma estradiol concentrations were not significantly different among treatment groups (F(3,19)=1.05, p=0.4; Fig. 5.1a). Plasma concentrations showed considerable variation with means ranging from 299 ± 96 to 863 ± 206 pg/ml. Plasma progesterone concentrations were also not significantly different among treatment groups (F(3,18)=0.88, p=0.5; Fig. 5.1b). Concentrations did not reach 10 ng/ml in the progesterone treated females (to mimic values obtained in wild, late vitellogenic/ovulatory females), despite initial trials.

Weight Analysis and Morphometrics

Mean wet weights of oviductal tissue were significantly different between treatment groups (F(3,19)=5.47, p=0.009; Fig. 5.1c). Wet weight of oviductal tissue in progesterone-treated females was significantly greater than for both estradiol-treated groups, but not for the vehicle-only treatment group. In the uterine tube and uterus, epithelial cell height was not significantly different among the four treatment groups (p > 0.05, Figs. 5.2b,c). The infundibulum, however, did show a significant difference between treatment groups (F(3,199)=5.77, p=0.0071; Fig. 5.2a). Post-hoc tests (both Scheffe and Bonferroni) indicate that the 6-week estradiol treated group is significantly different from the vehicle-only and 4-week estradiol treated group, but not the progesterone treated group. No significant regression was observed between plasma estradiol concentrations and epithelial cell height.
Figure 5.1. A: Mean plasma estradiol concentrations and B: mean plasma progesterone concentrations following treatment with vehicle solution only, estradiol for four or six weeks, or estradiol for four weeks followed by progesterone for two weeks. Plasma concentrations for individual animals are shown to the right of bars. Some points are obscured by other points of equal value. Horizontal lines represent minimum detectable concentrations. C: Mean oviductal weight as a percentage of total body weight in ovariectomised *Hoplodactylus maculatus* following treatment with vehicle solution only, estradiol for four or six weeks, or estradiol for four weeks followed by progesterone for two weeks. Lower case letters indicate treatment groups significantly different ($p<0.05$) from other groups. Bars that do not have letters in common differ significantly. Number in the box at the base of bars represents sample size. Error bars are standard errors.
Estradiol and progesterone effects

**A. Estradiol**

**B. Progesterone**

**C. Oviduct weight**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vehicle</th>
<th>Estradiol 4 weeks</th>
<th>Estradiol 6 weeks</th>
<th>Progesterone</th>
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<tr>
<td>Concentration (pg/ml)</td>
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<tr>
<td>Concentration (ng/ml)</td>
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<tr>
<td>Oviduct Weight (%)</td>
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Figure 5.2. Mean epithelial cell height in the A: Infundibulum, B: Uterine Tube, and C: Uterus of individual, ovariectomised *Hoplodactylus maculatus* following treatment with vehicle solution only, estradiol for four or six weeks, or estradiol for four weeks followed by progesterone for two weeks. Lower case letters indicate treatment groups significantly different (p<0.05) from other groups. Bars that do not have letters in common differ significantly. Error bars are standard errors.
in the three regions examined (infundibulum: \( R^2=6.9\%, \text{d.f.}=1,18, F=1.34 \); uterine tube: \( R^2=1.8\%, \text{d.f.}=1,18, F=0.33 \); uterus: \( R^2=15.4\%, \text{d.f.}=1,18, F=3.28 \)).

**General Morphology and Ultrastructure**

Unexpectedly, no differences were obvious at the light or electron microscopy level among the different treatment groups. There was, however, variation between individual females not particular to any treatment. General structure of the oviduct, with the distinct regions (infundibulum, uterine tube, isthmus, uterus, vagina) and layering within regions (mucosa, muscularis, serosa, Fig. 5.3), was consistent with that reported in wild vitellogenic *Hoplodactylus maculatus* (Chapter 2) and will not be discussed further. Structure and ultrastructure of the infundibulum, uterine tube, and uterus are described below. Descriptions apply to all treatment groups.

Nucleoli were prominent in the nuclei when tissues were cut in the appropriate section. Most nuclei appeared to be in interphase with peripheral chromatin common (Fig. 5.6d). Ciliated cells present in each region shared similar characteristics. Cilia were interspersed with microvilli. The cytoplasm contained numerous small, supranuclear mitochondria, free ribosomes and polyribosomes. Nuclei were situated basally.

Material characteristic of lipid was observed in several females (Figs. 5.4a,c,d). This material was situated predominantly, but not exclusively, at the basal region of epithelial and glandular cells, near the basement membrane. The lipid ranged in size from small droplets to quite large accumulations. A feature noted in some females (examples from all treatment groups) were aggregations of electron dense particles surrounding the presumed lipid material (Fig. 5.4c). These were seen most extensively in the infundibulum. In the cells of some individuals, the cytoplasm contained areas of amorphous material which did not contain any organelles (Figs. 5.5b, 5.6c).

**Infundibulum**

The epithelium in the infundibulum was low with differentiation into ciliated and non-ciliated cells (Fig. 5.3a,b,c). Non-ciliated cells contained variable numbers of secretory granules of varying electron density (Fig. 5.4). Differences in electron density of secretory granules presumably relates to their carbohydrate
Figure 5.3. Light micrographs of the oviduct in ovariectomised *Hoplodactylus maculatus*. Infundibulum from a female treated with A: vehicle solution only, B: estradiol for six weeks, and C: estradiol for four weeks followed by progesterone for two weeks. Uterine tube from a female treated with D: vehicle solution only, E: estradiol for six weeks, and F: estradiol for four weeks followed by progesterone for two weeks. Uterus from a female treated with G: vehicle solution only, H: estradiol for six weeks, and I: estradiol for four weeks followed by progesterone for two weeks. e: epithelium, g: gland, l: lumen, m: muscularis, z: connective tissue. Scale bars = 50 μm.
Estradiol and progesterone effects
Figure 5.4. Transmission electron micrographs of the infundibulum from ovariectomised *Hoplodactylus maculatus*. Epithelial tissue from a female treated with A: vehicle solution only, B: estradiol for four weeks, C: estradiol for six weeks, and D: estradiol for four weeks followed by progesterone for six weeks. c: ciliated cell, f: lipid droplet, l: lumen, n: non-ciliated cell, s: secretory granules. Scale bars = 5 μm.
Estradiol and progesterone effects
staining properties. There was no positive staining with Alcian blue in the epithelial layer; however, varying amounts of PAS-positive staining for carbohydrate were noted in the apical tips of some non-ciliated cells. Some non-ciliated cells formed blebs into the lumen (Figs. 5.4a,b,d). Interdigitation of cellular membranes was common at the lateral surfaces (Fig. 5.4). Aggregations of electron-dense particles were extensive within the infundibulum of some individuals (not particular to any treatment group). They were often seen surrounding presumed lipid droplets, as well as around the nuclei, and sometimes appeared to be coalescing (Fig. 5.4c). Golgi apparatus and rough endoplasmic reticulum (RER) were not obvious in cells of the infundibulum, although there were numerous free ribosomes.

**Uterine Tube**

The mucosa of the uterine tube was lifted into longitudinal folds (Fig. 5.3d,e,f) with alveolar glands often present at the base of these folds. The epithelial layer was differentiated into ciliated and non-ciliated cells. Non-ciliated cells, particularly at the apical region, stained positively with both PAS for carbohydrate and Alcian blue for acid mucosubstances. Staining intensity varied along the length of the uterine tube which may correspond with differences in the number and type of secretory granules seen in non-ciliated cells using the electron microscope (Figs. 5.5a,b,d). Granules were usually electron dense, although granules of low electron density were observed also. RER and Golgi apparatus were apparent in both epithelial and glandular cells, in addition to numerous free ribosomes.

There was no positive carbohydrate staining with either Alcian blue or PAS in gland cells. Glands contained secretory granules of medium electron density as well as granules which appeared to be in the process of development (Fig. 5.5c). The cytoplasm in some cells showed signs of disintegration. Membranes surrounding gland cells exhibited areas of interdigitation. In the glands of some individuals, clusters of droplets characteristic of lipid were noted at the basal edge of cells (Fig. 5.5c).

**Uterus**

The epithelial layer of the uterus was generally of an even height with a mix of columnar ciliated and non-ciliated cells (Fig. 5.3g,h,i). Non-ciliated cells stained
Figure 5.5. Transmission electron micrographs of the uterine tube from ovariectomised *Hoplodactylus maculatus*. A: Epithelial tissue from a female treated with vehicle solution only, B: Epithelial tissue from a female treated with estradiol for four weeks, C: Glandular tissue from a female treated with estradiol for six weeks, and D: Epithelial tissue from a female treated with estradiol for four weeks and progesterone for two weeks. a: amorphous material, c: ciliated cell, f: lipid, l: lumen, q: nucleus, s: secretory granules, t: connective tissue. Scale bars = 5 μm.
faintly with Alcian blue for acid mucosubstances, but strongly with PAS for carbohydrate (particularly the apical half). The staining corresponded to areas within the cells which contained numerous secretory granules (Figs. 5.6a,c,d). Granules were interspersed with other organelles including Golgi apparatus, RER and free ribosomes. Numerous blood vessels (Fig. 5.6d) lay just beneath the epithelial layer along the entire length of the uterus. In certain individuals (not particular to any treatment group), uterine epithelial cells contained large areas with little or no cytoplasm (Fig. 5.6a). This may, however, correspond to tissue damage due to TEM processing as it was not noted at the light microscopy level.

Gland cells contained secretory granules of varying electron density as well as some granules in the process of formation (Fig. 5.6b). Granules were concentrated in the apical region of glandular cells. Numerous RER were present, along with Golgi apparatus. Clusters of droplets characteristic of lipid were present in some cells on the outer perimeter of glands. The cytoplasm of some glandular cells looked degraded.

DISCUSSION

The results obtained in this experiment were unexpected in several respects. No differences in oviductal structure were observed between the four groups of ovariectomised gecko (Hoplodactylus maculatus) treated with estradiol (4 or 6 weeks), estradiol and progesterone, or vehicle solution only, nor did plasma estradiol or progesterone concentrations vary among treatments in an expected manner.

Initially, females were ovariectomised to remove the natural source of estradiol. It was expected that oviductal tissue would show signs of regression (for instance: a reduction in cell height, de-differentiation and reduced secretory activity in epithelial and glandular tissue), as has been reported in previous studies concerning both reptiles (I. Callard et al., 1972b; Yaron, 1972; Mead et al., 1981) and other vertebrates (Verhage and Brenner, 1975; Bareither and Verhage, 1980, 1981; Verhage et al., 1990). In H. maculatus, however, no regression of oviductal tissues was noted in the vehicle-only group. The oviducts of vehicle-only females appeared fully secretory with no obvious differences from other treatment groups. However, once plasma estradiol concentrations were considered, it was not surprising that no oviductal regression was observed.
Figure 5.6. Transmission electron micrographs of uterine tissue from ovariectomised *Hoplodactylus maculatus*. **A:** Epithelial tissue from a female treated with vehicle solution only (Black arrow: large gaps in tissue), **B:** Glandular tissue from a female treated with estradiol for four weeks (small black arrow: interdigitation of cell membrane), **C:** Epithelial tissue from a female treated with estradiol for six weeks, and **D:** Epithelial tissue from a female treated with estradiol for four weeks and progesterone for two weeks. a: amorphous material, c: ciliated cell, f: lipid, l: lumen, n: non-ciliated cell, q: nuclei, s: secretory granules, t: connective tissue, v: blood vessel. Scale bars = 5 μm.
Estradiol and progesterone effects
Estradiol concentrations were high and variable in all treatment groups (range: 134-2230 pg/ml), including the vehicle-only females (467 ±163 pg/ml). In other studies, ovariectomy has significantly reduced plasma estradiol concentrations (for instance, <100 pg/ml in the snake *Thamnophis elegans*, Mead et al., 1981). The reason for the unexpectedly high and variable estradiol concentrations is unknown.

In wild female *H. maculatus* analysed to date, plasma estradiol concentrations have not exceeded 150 pg/ml except in two vitellogenic females (>2000 pg/ml; Girling, 1993; A. Cree, pers. comm.). In other species, the highest plasma concentrations of estradiol are commonly measured during vitellogenesis, dropping again following ovulation (for instance: I. Callard et al., 1978; S. Jones and Swain, 1996; Sarker et al., 1996; S. Jones et al., 1997). For example, in the snake *Thamnophis elegans*, plasma concentrations of estradiol in vitellogenic females were reported to be approximately 1800 pg/ml, dropping to less than 100 pg/ml during pregnancy (Mead et al., 1981).

Individual females were labelled twice using a toe-clip and a number drawn on their back (the skin did not shed over the course of the experiment). This provided a double check of identify prior to the hormone injections being given. Blood and tissue samples were all labelled with the individual female's number and the date as samples were collected. Thus, it is very unlikely that confused labelling caused the unexpected results. Additionally, no ovarian tissue remained following ovariectomy. This was confirmed on dissection of females at the completion of the experiment.

Possible contamination of vehicle-only females with estradiol, although unlikely, can not be fully excluded. A single glass syringe (with new needle for each female) was used to allow accurate administration of the volume required. This was thoroughly rinsed (using distilled water) between use with different solutions, but it is possible that residual amounts of estradiol were injected into vehicle-only females. Even if contamination occurred, however, the amount of estradiol received by vehicle-only females would have been insignificant and that this volume should suffice to promote the estradiol concentrations observed after ovariectomy is highly unlikely. Other factors to consider include the clearance rate of estradiol from females following ovariectomy, and other potential extra-ovarian sources of estradiol in *H. maculatus*. 
To my knowledge, no one has analysed the clearance rate of estradiol from the body of a reptilian species, although it has been considered for other steroid hormones. For example, in the lizard *Lacerta vivipara*, the half-life of tritiated corticosterone in plasma was four times lower in hibernating ($t_{1/2} = 8$ h) versus non-hibernating ($t_{1/2} = 2$ h) females. This difference was related to a slower elimination of radioactivity from plasma and a reduced metabolism of corticosterone during hibernation (Dauphin-Villemant et al., 1990). To fully understand the actions of estradiol in *H. maculatus*, the clearance rate of estradiol from the gecko’s body under natural conditions should be determined, as well as the possibility that clearance rate is altered by ovariectomy, and other stressful conditions. Clearance rate should be considered alongside potential extra-ovarian sources of plasma estradiol as it is highly unlikely that a even a reduced clearance rate could maintain the high plasma estradiol concentrations after the two-three month recovery and experimental period.

Other possible extra-ovarian sources of plasma estradiol must also be considered, including the production of androgens which could be further aromatased to estradiol. The adrenal gland, for instance, is worthy of further attention. Although the major hormones secreted by the reptilian adrenal gland are corticosteroids (Dauphin-Villemant and Xavier, 1985), the adrenal has also been reported to produce androgens (Tam et al., 1972). In the cobra, *Naja naja*, levels of androgens produced were low and did not show any seasonal variation (Tam et al., 1972). However, potential secretion of androgens by the adrenal of female *H. maculatus* and other gecko species has not been investigated.

Conversion of androgens to estrogen (aromatisation) is known to occur in the brain and pituitary glands of vertebrates, including reptiles (G. Callard et al., 1977; G. Callard et al., 1978; Lisboa et al., 1978; G. Callard, 1983; Staub and DeBeer, 1997). In several teleost species, estrogen production by the gonads was relatively low when compared to brain tissues (weight for weight, G. Callard, 1983). Experimental conditions may also change the amount of aromatisation occurring in the brain. In adult (male and female) rabbits, castration was associated with an increase in aromatisation by brain tissues *in vitro* (Reddy et al., 1973). Estrogens synthesised by the brain are believed to reach the bloodstream in songbirds (discussed in Schlinger, 1994), however, whether this occurs in reptiles is unknown.
Estrogen can also be derived from aromatisation of circulating androgens in other tissues such as bone, skin and fat (Siiteri et al., 1982). The oviduct itself is also a potential site of estrogen production, as well as a site of direct action by androgens. Androgen receptors and aromatase activity were detected in the oviduct of the turtle Trachemys scripta (S. Smith et al., 1995). Androgens are known to stimulate the oviduct in both ovariectomised and intact regressed female Hemidactylus flaviviridis (Prasad and Sanyal, 1969).

The oviduct may also be influenced by factors other than estrogens or androgens. In the lizard, Lacerta sicula, for instance, administration of pregnant mare serum (PMS; contains an equine placental gonadotrophin), to intact, non-reproductive females caused development of the oviduct (Botte and Basile, 1974). However, in ovariectomised females, PMS did not modify morphological structure of the oviduct, but did induce some increase in DNA, protein content and alkaline phosphatase activity. This suggests that gonadotrophins may act directly or indirectly on the oviduct without interaction with ovarian hormones such as estradiol.

After ovariectomy and associated disruption of feedback loops, adrenal and other tissues may well be an important source of sex steroids (G. Callard, 1983). Further experimental work is required to clarify these issues, particularly in the light of such unexpected results in this experiment. The effect of ovariectomy on oviductal tissue needs to be determined, without further experimental treatment. Concentrations of estradiol should be analysed prior to surgery, as well as periodically during the recovery period. This was not possible in this study due to the small body size of the geckos making repeated blood sampling unfeasible. It would be interesting also, to determine the clearance rate of estradiol from wild females over several reproductive stages, as well as following ovariectomy. The potential for other body tissues (brain, pituitary, fat, oviduct) to produce both androgens and estrogens needs to be considered.

No additional effects of continued injections with estradiol from four - six weeks in comparison to vehicle-only females were noted in the oviduct. Maturity of cells may well be reached prior to hormone treatment (in vehicle-only females) and hence any further development would not be expected.

No differences in oviductal structure were noted in estrogen-primed, progesterone treated females in comparison to other experimental groups. This
result is consistent with certain other microscopy studies administering progesterone to reptilian species (Prasad and Sanyal, 1969; Mead et al., 1981), despite the sequence of progesterone administration differing in my study (progesterone administered to estrogen-primed females). Results from progesterone studies, however, have varied considerably. Both progesterone and estradiol were required to restore the oviduct to normal in the viviparous lizard, Xantusia vigilis (ovariectomised, pre-ovulatory females; Yaron, 1972). Similarly, estradiol and progesterone were reported to work synergistically to cause proliferation of the oviductal mucosa in juvenile Chamaeleo pumilus pumilus (Veith, 1974). In the same species, progesterone administered to gravid, ovariectomised females caused an increase in the blood supply to the oviduct (Veith, 1974). In the turtle, Chrysemys picta, progesterone administered to pre-ovulatory females caused a significant decrease in oviduct weight (Klicka and Mahmoud, 1977). Similarly, in mid-vitellogenic iguanid lizards, Sceloporus cyanogenys, progesterone inhibited oviductal growth (I. Callard et al., 1972a).

It appears the wide variety of results relates to the reproductive condition of animals chosen and whether surgical manipulation is used. The different results obtained suggest progesterone may act in a complex manner in combination with the hormone milieu present in the animal at the time. For instance, in the chick oviduct, the effect of progesterone differs depending on the stage of oviduct development (Oka and Schimke, 1969). In an immature oviduct, progesterone prevents estrogen-induced formation of tubular gland cells. However, once tubular gland cells have formed (after treatment with estrogen), progesterone can be substituted for estrogen in maintaining the function of cells. If estrogen treatment is withdrawn after estrogen-induced development of tubular gland cells has occurred, gland cells cease to function. The cessation in function is indicated by a stop in lysozyme and ovalbumin production. Both progesterone and estrogen can re-stimulate the function of these cells (Oka and Schimke, 1969).

The results obtained in this experiment, however, prevent unequivocal interpretations about the effects of progesterone. High plasma estradiol concentrations in all treatment groups and an apparent lack of oviductal regression in vehicle-only females were unexpected and potentially confound any results obtained following progesterone treatment. Additionally, I do not know why plasma progesterone did not vary between treatment groups and why it did not reach the levels recorded in females from the original trial. The experiment was designed to mimic progesterone patterns recorded in the wild,
with progesterone administered to estrogen-primed females. This experimental design should be reconsidered once the mechanisms behind the unexpected results are determined.

Oviductal tissues from this experiment resembled tissues that were analysed from wild, vitellogenic females (Chapter 2). Epithelial cell heights measured in this experiment were very similar to the heights measured in late vitellogenic females, as were the structure and contents of glandular and epithelial cells. Although the general structure and ultrastructure of the oviduct in females from this experiment was similar to wild, vitellogenic females (Chapter 2), certain differences were noted. Material characteristic of lipid was prominent in all regions examined, particularly the infundibulum. The reason for this is unknown, although it may reflect dietary factors. Geckos were provided with fruit \textit{ad libitum} as well as insects periodically. This stable food source may be greater than what is available in their natural diet allowing additional fat storage. Extensive lipid material was also noted in oviductal tissue from captive females which were used to trial different electron microscopy techniques (unpubl. obs.).

A further difference noted in certain individuals (examples in all treatment groups) were aggregations of electron dense particles, particularly around lipid deposits. The nature of these particles is not known. No differences were noted between slides treated with or without the saliva test prior to PAS staining suggesting that the particles are not glycogen (Humason, 1979).

In conclusion, there was no significant difference in plasma concentrations of estradiol and progesterone among the different treatment groups. Plasma estradiol concentrations were high and variable in all treatment groups, including females receiving vehicle solution only. There were no differences in oviductal structure among the four treatment groups, oviducts resembled those observed in wild, vitellogenic females. These unexpected results need further consideration and additional experimental work is required to determine why results do not follow the pattern observed in similar experiments in other reptilian and vertebrate species. No oviductal changes were noted following progesterone treatment in estrogen-primed ovariectomised females. These results, however, are confounded by the unexpected plasma estradiol and progesterone concentrations. This experimental design should be reconsidered once the above mentioned findings are explained.
Chapter Six

Detection of insulin-like growth factor-I in the gekkonid oviduct using immunocytochemistry

Page

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INTRODUCTION

A major research area in recent years has been the isolation and analysis of a wide variety of growth factors and cytokines which may act in an endocrine, paracrine or autocrine fashion to regulate growth and development throughout an organism's ontogeny. As yet, however, work has focussed on mammalian species and little is known concerning growth factors and their role in reptilian species.

Numerous growth factors have been identified in the mammalian reproductive tract (for instance: epidermal growth factor [EGF], insulin-like growth factors [IGF], transforming growth factors) and these have been implicated in various functions (Simmen and Simmen, 1991; Murphy and Barron, 1993; S. Smith, 1994; Tabibzadeh, 1994). In reptiles, IGF-I (Palmer and Guillette, 1991; Cox and Guillette, 1993), EGF (Palmer and Guillette, 1991) and interleukin-I (Paulesu et al., 1995) have been identified in oviductal tissue. The roles these growth factors play in reproduction are just beginning to be determined (see Chapter 7).

In mammalian species, the actions of estradiol are believed to be facilitated by growth factors. For instance, in mice, estradiol stimulates uterine and vaginal growth in vivo. However, to elicit the same epithelial differentiation and hypertrophy in vitro, epithelial tissue must be cultured in combination with stromal tissue (Cooke et al., 1986) suggesting paracrine growth factors are involved. This hypothesis was supported by work by Nelson et al. (1991) who showed that EGF administered to ovariectomised mice acted as a potent uterine and vaginal mitogen. Additionally, an antibody specific for EGF significantly inhibited estrogen-induced uterine and vaginal growth (Nelson et al., 1991). In reptiles also, it appears growth factors play a role in oviductal development. In the gecko Hemidactylus turcicus, both IGF-I and EGF induced hypertrophy of the oviduct in ovariectomised females, although not to the same extent as exogenous estradiol (Cox, 1994).

Although the reptilian forms of IGF-I have yet to be purified and characterised, IGF-I like immunoreactivity has been reported in the uterine tube and uterus of the alligator Alligator mississippiensis and the tortoise Gopherus polyphemus (Palmer and Guillette, 1991; Cox and Guillette, 1993). IGF-I has also been detected in the plasma of A. mississippiensis (Crain et al., 1990) with maximal levels
observed during gravidity correlating with high plasma progesterone concentrations (Guillette et al., 1996).

In the initial design of an experiment examining the effects of estradiol on the oviduct of the oviparous gecko, *Hemidactylus turcicus* (Chapter 4), immunocytochemistry to identify the presence of IGF-I in oviductal tissue was to be performed. In the experiment, ovariec tomised females were treated with estradiol and the development of uterine tissues was analysed over a two week period. One oviduct from each female was dissected out and used for electron microscopy, the other was preserved for light microscopy and immunocytochemistry. I hoped to identify which part(s) of oviductal tissues produced IGF-I and how this distribution might change over the two week period. I hypothesised that the production of IGF-I would increase as estradiol treatment progressed. I expected IGF-I to be distributed predominantly in the epithelium and mucosal glands where much of the development is seen. Unfortunately, an accident by one of the technical staff meant that the majority of tissues preserved for immunocytochemistry were destroyed. This prevented a major part of this experiment from being completed.

I still hoped to validate the immunocytochemistry technique and use it to identify whether IGF-I was present in the oviductal tissues of *Hoplodactylus maculatus* which were analysed over a reproductive cycle (Chapter 2). I hypothesised that IGF-I would be found in the oviduct associated with the developing epithelium and mucosal glands present during vitellogenesis. However, despite considerable effort, I had problems completing satisfactory validations and I therefore decided, after consultation with my supervisors and committee, not to continue with this part of my research in view of the additional time and expense that would be involved. The methods used and the results obtained are presented here in summary form to facilitate future research in this area.

**METHODS**

Oviductal tissues from *Hoplodactylus maculatus* were fixed in Bouins, stored in 70% ethanol, embedded in wax blocks, and cut and mounted on glass slides as for light microscopy (see methods, Chapter 2). I used a Vectorstain Elite ABC (avidin-biotin conjugate) kit purchased from Vector Laboratories (Burlingame,
IGF-I in the gecko oviduct

CA). Briefly, sections were deparaffinised in xylene and alcohol, then incubated in either primary antibody (polyclonal rabbit anti-human IGF-I, provided courtesy of the National Hormone and Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD), primary antibody pre-absorbed with human IGF-I, rabbit immunoglobulins (IgG), or buffer solution. Sections were then washed in buffer solution, incubated in biotinylated secondary antibody (goat anti-rabbit IgG), washed, incubated in ABC reagent (avid and biotin horseradish peroxidase conjugate), washed, and then the labelled tissue was visualised using diaminobenzidine tetrachloride (DAB) which stains tissues a chocolate brown colour.

In addition to gecko oviductal tissues, mouse uterine tissue from pregnant females (day three) was also processed and treated in the same manner. These tissues are known to show positive IGF-I staining with the same antibody (Kapur et al., 1992).

The protocol used was the same as that used successfully in the laboratory of Professor L.J. Guillette, Jr. (Cox and Guillette, 1993). Other published studies have used variations on similar protocols, often using one of the commercially available kits (for example: Kapur et al., 1992).

RESULTS AND DISCUSSION

All gecko oviductal tissue undergoing this procedure stained brown (Fig. 6.1a). This included the epithelium (especially the apical surface of ciliated cells, i.e. edge effects), glandular tissue, muscularis and the serosa. Connective tissue stained only lightly. Additionally, in some females analysed, the whole body was processed with the oviduct left in situ. All tissues stained brown including gut epithelium, muscle, etc. Tissues which were treated without primary antibody (buffer solution only) did not stain (Fig. 6.1b), confirming that the secondary antibody did not react with tissue sections. However, tissues treated with rabbit IgGs, or with anti-IGF-I pre-absorbed with human IGF-I, also stained in the same way (Fig. 6.1c). This suggests that the staining is not specific to IGF-I and represents background staining. As the secondary antibody did not react, it appears that the problem lies with non-specific binding by the primary antibody.
Figure 6.1. Uterine tube from late vitellogenic *Hoplodactylus maculatus* processed for immunocytochemistry. **A:** Tissue incubated with polyclonal rabbit anti-human IGF-I. **B:** Control tissue incubated with buffer solution only. **C:** Control tissue incubated with rabbit IgG. black arrow: ciliated cell, white arrow: melanin, e: epithelium, g: gland, l: lumen, m: muscularis.
IGF-I in the gecko oviduct
Uterine tissue from pregnant female (day three) mice is known to show positive IGF-I staining with the same antibody (antibody concentration 1:1000; Kapur et al., 1992). However, I did not observe the pattern of staining seen by Kapur et al. (1992). Positive staining was only seen when high antibody concentrations were used (1:100), but all tissues stained, not specific regions as determined by Kapur et al. (1992).

There are various reasons why the expected pattern of staining was not observed in the mouse uterine tissues (and gecko tissues also). The most likely of these is that fixation and processing of the tissue has damaged the antigens within the tissues. Once tissues were cut and mounted on slides they were stored (often for many months) in covered trays. Tissue degeneration may have occurred on aging mounted sections, so I cut fresh tissue (mouse uterus) from old wax blocks and analysed as above. There was no difference in the staining pattern observed. This does not mean that the age of processed tissues is not a problem, but that the initial problem (lack of specific staining in mouse uterine tissues) is not related to the age of the sectioned tissues. Deterioration may still have occurred within the wax blocks prior to sectioning. Determining the reason for non-specific staining will require further testing, and in all likelihood, fixation of new tissues, possibly using freezing techniques, to minimise loss of antigenicity.

While attempting to solve the problems encountered during the immunocytochemistry, considerable reading (Leong, 1993; Taylor, 1994; Taylor and Tandon, 1994) has increased my understanding of the technique, and its limitations. I believe the use of immunocytochemistry to identify antigens such as growth factors will be a useful technique in reptilian reproductive biology (see Chapter 7). However, it will be a more appropriate technique to use in conjunction with other methods which identify and analyse characteristics of the factor of interest.
Chapter Seven

Structure And Function of the Reptilian Oviduct

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ABSTRACT

This review details information available concerning the structure and functions of the oviduct of reptiles, and the mechanisms controlling these functions. The review incorporates research discussed in previous chapters of this thesis.

The oviduct, which is derived from the embryonic Müllerian duct, can be divided into several different regions which differ in form and function. Starting anteriorly, the infundibulum initially receives the egg following ovulation. The infundibulum has a secretory epithelium, but the nature of the secretory material is unknown. The egg travels down the uterine tube where, in crocodilians and turtles, the albumen layer is secreted by mucosal glands (no albumen layer is present in lepidosaurians). The egg then passes through an intermediate region called the isthmus and then into the uterus. In the uterus of oviparous species, components of the eggshell are secreted. The hormonal mechanisms controlling eggshell production are unknown. In viviparous species, placental interactions form between uterine tissues and extra-embryonic membranes. Little is known about nutrient transfer across the placenta, nor potential endocrine interactions between maternal and foetal tissues. At the completion of gravidity/gestation, the egg passes through a muscular vagina and the common urogenital sinus before egg-laying/birth. The major hormones involved in egg-laying or birth include AVT and prostaglandins. However, the interactions between these hormones and other neural factors are still being determined. Sperm may be stored in sites in the vagina and/or anterior oviductal regions. As yet, we do not know if, or how, the oviduct contributes to sperm maintenance during storage. Fertilisation is believed to occur in the anterior regions of the oviduct.

The oviduct undergoes seasonal changes in structure which can be correlated with seasonal patterns in plasma hormone levels. Estradiol appears to be the main influence controlling the development of the oviduct during vitellogenesis. Other hormones, including gonadotropins, androgens, progesterone and prolactin, may also act on oviductal tissues. Surgical and hormonal manipulations have been used on various occasions to analyse the actions of hormones on oviductal tissues. Hypertrophy of the oviduct can be induced if exogenous estradiol is administered to ovariectomised females. That sex-steroid hormones act directly on oviductal tissues is implied by the presence of sex-steroid receptors (estrogen, progesterone and androgen receptors), as well as various steroidogenic and metabolic enzymes. A developing area of research is
the identification of various growth factors and their actions within oviductal tissues. To date EGF, IGF-I and -II and IL-I have been identified in the reptilian oviduct. EGF and IGF are believed to act as facilitators of estradiol-induced proliferation. IL-I is believed to act in placentation.

Despite recent developments in our understanding of the different oviductal regions and their functions, there are still large gaps in our knowledge concerning oviductal structure and function. The improved techniques now available to identify endocrine, molecular and biochemical mechanisms will be invaluable to increase our understanding of the oviduct and its actions. Certain key areas for future research have been identified and include the actions of androgens on oviductal tissues, the analysis of growth factors and their actions within the oviduct, the mechanisms of calcium secretion during egg-shelling, the potential endocrine interactions between placental tissues, and the differential response of varying oviductal regions to hormones and neural factors involved in birth and egg-laying.
INTRODUCTION

The reptilian oviduct, which includes all structures derived from the embryonic Müllerian duct (Wake, 1985), is a fascinating organ with multiple functions. The oviduct acts as a conduit for the egg between ovulation and oviposition/parturition. It acts as a site for fertilisation and, in some species, sperm storage. It may provide an oocyte with albumen and a multi-layered eggshell. Alternatively, the oviduct may function with extra-embryonic membranes to form placental interactions providing gas and water exchange, and potentially nutrient transfer. The oviduct is a dynamic organ, developing and regressing with the changing reproductive seasons. The reptiles themselves exhibit variation in reproductive mode which is reflected in the oviduct and its functions. These functions, which range from eggshell production to placentation, mean the reptilian oviduct is intimately associated with the long-standing debates surrounding parity mode and nutrient provision to embryos.

Although the oviduct has long been a organ of interest, understanding of its actions is still in its infancy. This is especially apparent when compared with the advances made for other vertebrate groups, specifically the eutherian mammals. There are large gaps in the knowledge concerning a variety of issues, particularly relating to the endocrine control and molecular and biochemical nature of the oviduct.

There are four living orders of reptiles: Crocodilia (crocodiles, alligators, caimans and gavials), Sphenodontia (tuatara), Squamata (lizards and snakes) and Testudines (turtles and tortoises, which will collectively be called turtles in the following discussion). The Sphenodontia and Squamata make up the Lepidosauria. The aim of this review is to summarise knowledge to date related to oviductal structure and function in reptiles, including differences among the different reptilian orders. No thorough description is yet available concerning oviductal structure in tuatara so they are not considered in detail here. However, tuatara are believed to exhibit a typically reptilian oviduct with numerous uterine glands (Osawa, 1898; Gabe and Saint Giron, 1964; H. Fox, 1977). Areas in particular need of further research will be identified. H. Fox (1977) provided a detailed summary of early information available concerning the structure of the oviduct in reptiles. To avoid excessive repetition, information summarised here will predominantly use data which became available after 1977.
Terminology

Terminology concerning the oviduct varies in a potentially confusing manner, even warranting a paper discussing the relative merits of 'oviductal' versus 'oviducal' as the adjectival form of oviduct (H. Smith et al., 1989). H. Smith et al. (1989) concluded that 'oviductal' was the preferred form and, for the sake of consistency, this term will be used throughout this paper.

The oviduct can be divided into several different regions which differ in their structure and function (Fig. 7.1a). The names of these regions vary to some extent and this can cause confusion. Those terms underlined below will be used consistently throughout this paper. Starting anteriorly, the regions include: 1. infundibulum, 2. uterine tube (also known as the tuba, glandular region, albumen secreting portion, magnum), 3. isthmus (aglandular segment, intermediate region), 4. uterus (shell forming region) and 5. vagina (cervix). The different regions are not recognised in all reptilian species, and additional regions may also be included. In several squamate species, the infundibulum and uterine tube were not differentiated (Halpert et al., 1982; Adams and Cooper, 1988; Aldridge, 1992; Shanthakumari et al., 1992) and the uterus was divided into anterior and posterior regions (Guillette and Jones, 1985b; Guillette et al., 1989). In the crocodilians studied to date, the infundibulum and the uterus were each separated into anterior and posterior portions (Palmer and Guillette, 1992). The different regions will be discussed in more detail below.

The oviduct can also be differentiated into different tissue layers in cross section (Fig. 7.1b). The innermost layer or mucosa (endometrium) consists of an epithelial layer lining the lumen of the oviduct plus its underlying lamina propria. The lamina propria consists of connective tissue and any glands which may be present. Under the mucosa is the muscularis (myometrium) which consists of an inner circular and an outer longitudinal muscle layer. The outermost layer or the serosa (perimetrium) is continuous with the peritoneum. Obviously, these layers differ in structure between the different oviductal regions. The terms endometrium, myometrium and perimetrium more correctly relate specifically to uterine tissues, but are commonly used to describe oviductal tissues in general in the reptilian literature. In this review, I will use mucosa, muscularis, and serosa for all regions to avoid confusion.
Parity Mode and Nutrient Provision to Embryos

In the following discussion, it will be necessary to highlight differences in oviduct structure and function which directly relate to the parity mode, or the method of nutrient provision to embryos, of the species in question. In the context of this discussion, oviparity is defined as the laying of eggs containing embryos which require a period of development outside of the female's reproductive tract (Guillette, 1993). Viviparity, on the other hand, is defined as the retention of the embryo within the uterus of the mother until development is complete and birth can occur (Guillette, 1993). The term gravid refers to oviparous females containing oviductal eggs. The term gestation refers to period of time when embryos are within the oviduct of viviparous females prior to birth.

Viviparity is believed to have evolved within the squamate reptiles over 100 times (Blackburn, 1982; Blackburn, 1985; Shine, 1985). However, within the crocodilians, turtles and sphenodontians, all species are oviparous. The ecological and physiological factors concerned with the evolution of viviparity in squamate reptiles have been widely debated (G. Packard et al., 1977; Angelini and Ghiara, 1984; Shine and Guillette, 1988; G. Packard et al., 1989; Shine, 1989; I. Callard et al., 1992; Guillette, 1993). The hypothesised sequence of physiological and morphological changes allowing the evolution of viviparity can be summarised as follows (G. Packard et al., 1977; I. Callard et al., 1992; Guillette, 1993): to allow viviparity to evolve, the embryo must be retained within the uterus of the mother for longer and longer time periods. Whilst in the mother, there is a need for increased gas and water exchange, hence the evolution of a placenta. The evolution of a placenta requires a reduction in eggshell thickness to allow closer association of uterine and embryonic tissues. Although the sequence is only summarised briefly, it is obvious already that the evolution of viviparity in reptilian species is intimately associated with changes in oviduct structure and function. Therefore, throughout this review the information discussed will be vital to considerations concerning the evolution of viviparity.

Two sources of nutrient provision to embryos are seen in reptiles. In the first of these, lecithotrophy, embryos receive their nutrients predominantly from the yolk supplied during vitellogenesis. Most reptiles, both viviparous and oviparous, exhibit lecithotrophy. The supply of nutrients by predominantly extra-vitelline means, such as via a placenta, is termed matrotrophy, and it is
exhibited in only a few reptilian species studied to date (Blackburn et al., 1984; M. Thompson and Stewart, 1994).

**STRUCTURE OF THE OVIDUCT**

The reptilian oviduct includes all structures of the female reproductive tract derived from the embryonic Müllerian duct (Wake, 1985). In general, the oviducts are paired structures, one lying dorsally on either side of the body (H. Fox, 1977). However, in certain species, one oviduct has been lost. For example, the left oviduct is absent in the skink Lipinia rouxi (Greer and Mys, 1987), and in the snake Tantilla coronata, the left oviduct is vestigial although females do have a functional left and right ovary (Aldridge, 1992). The oviducts may be different lengths on each side of the body (Perkins and Palmer, 1996), presumably to make efficient use of body space during gravidity or gestation. Below is a summary of structure of the different oviductal regions during the vitellogenic period when oviducts are fully developed in preparation for gravidity/gestation.

*Infundibulum*

The most anterior region of the oviduct, the infundibulum, tends to be a slender, flaccid region. It receives the ovulated egg from the ovary via a funnel-shaped ostium opening to the coelomic cavity. Prior to ovulation, the infundibulum migrates toward the ovary where the ostium surrounds the developing oocyte. This process has been observed in several squamate species (O. Cuellar, 1970) and means that the ostium is ideally placed to receive ovulated oocytes. Whether the oviduct is directly involved in stimulating the ovulation process is unknown.

The mucosal epithelium lining the lumen of the infundibulum extends to the tip of the ostium and back over onto the coelomic side of the tissue for a short distance where it merges with the serosal layer. The luminal epithelium in the most anterior portion of the infundibulum is made up predominantly of ciliated cells, changing to a more even mix of ciliated and non-ciliated cells more posteriorly. Underlying the non-glandular mucosa is a fine layer of smooth muscle, surrounded by the outer serosa. More posteriorly down the infundibulum, the mucosa may be thrown into folds of gradually increasing height towards the uterine tube (Botte, 1973; Palmer and Guillette, 1988; Uribe et al., 1988; Picariello et al., 1989; Palmer and Guillette, 1992; Sarker et al., 1995;
Perkins and Palmer, 1996; Chapters 2 and 3). In some species, the change in the mucosa is considered sufficient to divide the infundibulum into anterior and posterior regions (for example: *Gopherus polyphemus*, Palmer and Guillette, 1988).

The infundibulum is a secretory region. In the oviparous lizard *Sceloporus woodi*, deposition of secretory material began immediately upon entry of the egg into the oviduct (Palmer *et al.*, 1993). This was evident for an egg only halfway inside the ostium; material was already deposited on the egg, but only on the portion inside the oviduct. Non-ciliated cells of the epithelium may stain for carbohydrate or carbohydrate-protein substances with Periodic Acid-Schiff reagent (PAS; Botte, 1973; Guillette *et al.*, 1989; Picariello *et al.*, 1989; Chapters 2 and 3). In the gecko species *Hoplodactylus maculatus*, *Saltarius wyberba* and *Hemidactylus turcicus* (Chapter 3), the positive carbohydrate staining corresponded to numerous secretory granules of varying electron density in the apical regions of cells. Some non-ciliated cells exhibited apical protrusions into the lumen. In the case of *H. turcicus*, the nuclei were positioned within these protrusions giving the appearance that the cells were about to slough off into the lumen.

Bleb cells have been noted in several species. In the tortoise *Gopherus polyphemus*, bleb cells were found at the base of folds in the posterior infundibulum (Palmer and Guillette, 1988). These cells had a smooth apical surface and did not stain positively for carbohydrate. Bleb cells were also observed in the infundibulum of the geckos *H. maculatus*, *S. wyberba* and *H. turcicus* (Chapters 2 and 3). Using transmission electron microscopy, I suggested the most likely candidate for bleb cells in these geckos were cells with a fine, apical membrane protruding into the lumen. The protrusions contained fine, non-granular material and the cytoplasm below the bleb resembled other non-ciliated cells. In fact, numerous small blebs and other apical protrusions were noted on the apical surface of epithelial cells from all three gecko species examined (Chapter 3). The function of these cells is unknown, but Palmer and Guillette (1988) suggested for *G. polyphemus* that they may be involved in apocrine or merocrine secretion. Apical protrusions in the infundibulum are ideally placed to secrete materials directly onto the ovulated egg prior to the egg being surrounded by albumen and shell membranes.
In squamates, the mucosa of the uterine tube is formed into folds of connective tissue covered with epithelial cells (Botte, 1973; Uribe et al., 1988; Picariello et al., 1989; Palmer et al., 1993; Perkins and Palmer, 1996, Chapters 2 and 3). The epithelium lining the lumen of the uterine tube consists of both columnar ciliated and non-ciliated cells. The staining properties of the epithelial cells differs from those in the infundibulum. In most squamates analysed to date, non-ciliated cells stain positively for carbohydrate and carbohydrate-protein substances with PAS and for acid mucosubstances with Alcian blue. In *Lacerta sicula* (Botte, 1973) and the gecko species analysed in Chapter 3, tests indicate that the staining product is not glycogen. The bases of mucosal folds in the posterior uterine tube form glandular crypts (in both oviparous and viviparous species). Although epithelial cells lining the crypts do not stain positively with various carbohydrate stains, gland cells do contain numerous secretory granules. Under the mucosa are layers of both circular and longitudinal muscle which are bounded by the outer serosa.

The uterine tube of turtles and crocodilians differs in some ways from that of squamates. As in squamates, a simple columnar epithelium of ciliated and non-ciliated cells is present, the non-ciliated cells of which stain positively for carbohydrate substances (Palmer and Guillette, 1988; Abrams Motz and Callard, 1991; Sarker et al., 1995), but the mucosa is packed with numerous glands occupying 80-90% of the total thickness of the uterine tube. Glands may be compound tubulo-alveolar, branched tubular, or branched acinar (Palmer and Guillette, 1988; Abrams Motz and Callard, 1991; Sarker et al., 1995). In the alligator *Alligator mississippiensis*, gland cells contained spherical granules of varying electron density (Palmer and Guillette, 1992) and the surface of gland cells was covered with numerous microvilli.

**Isthmus**

As an intermediate region between the uterine tube and the uterus, the isthmus often appears to share similarities with both its neighbouring regions. It is also a region which is often short and undescribed. In the gecko species *H. maculatus*, *S. wyberba* and *H. turcicus*, the isthmus was not visible with the naked eye and it was morphologically similar to both the uterine tube and the uterus (Chapter 3). Unlike the epithelium of neighbouring regions, however, the epithelium of the
Isthmus did not stain with either PAS or Alcian Blue. In the gecko *Tarentola m. mauritanica*, the epithelium of both the isthmus and the uterine tube stained positively with both PAS and Alcian blue, and the mucosal glands of the isthmus resembled those in the uterine tube and the uterus (Picariello *et al.*, 1989). The isthmus of the tortoise *Gopherus polyphemus* was aglandular (Palmer and Guillette, 1988), whereas in *Lissemys p. punctata* tubular alveolar glands similar to those in the uterine tube were seen, but only at the time of ovulation (Sarker *et al.*, 1995).

**Uterus**

The typical uterus of an oviparous squamate has a columnar epithelium with both ciliated and non-ciliated cells (Guillette *et al.*, 1989; Palmer *et al.*, 1993; Perkins and Palmer, 1996; Picariello *et al.*, 1989; Uribe *et al.*, 1988; Chapter 3). Staining of non-ciliated cells for carbohydrate substances varies between species. Unlike that of the uterine tube, the epithelium of the uterus overlays a thick *lamina propria* containing numerous mucosal glands (tubulo-alveolar, tubular, branched saccular or branched acinar), the cells of which contain numerous secretory granules. Under the mucosa is an inner layer of circular and an outer layer of longitudinal muscle, which are bounded by the serosal layer.

The uterus of turtles is very similar to that described for oviparous squamates (see above; Aitken and Solomon, 1976; Palmer and Guillette, 1988; Abrams Motz and Callard, 1991; Sarker *et al.*, 1995). However, in the crocodilian *Alligator mississippiensis*, the uterus was divided into two functionally separate regions. In *A. mississippiensis*, the mucosal glands of the anterior uterus were branched tubular with short ducts connecting them to the lumen (Palmer and Guillette, 1992). Gland cells were cuboidal and contained numerous electron dense granules. The posterior uterus also had numerous glands in the mucosa. However, gland cells lacked the extensive distribution of electron dense granules (Palmer and Guillette, 1992).

In *Sphenomorphus fragilis*, a lizard exhibiting incipient viviparity (i.e., it lays eggs which hatch within hours of oviposition), the uterus contained very few, but well developed, mucosal glands (Guillette, 1992). This illustrates the difference in uterine structure which is seen with the transition from oviparity to viviparity. Viviparous squamates have very few glands within the mucosa, although the gland cells still contain secretory granules of varying electron
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densities. The uterus in viviparous species, as in oviparous species, has an epithelium made up of a mix of ciliated and non-ciliated cells (Guillette and Jones, 1985b; Chapters 2 and 3) and the mucosa is well vascularised with numerous blood vessels under the epithelial layer.

Histochemical properties and the numbers of secretory granules in glandular cells vary between different species of reptiles (for instance: Chapter 3). In the gecko Tarentola m. mauritanica (Picariello et al., 1989) and the lizard Lacerta sicula (Botte, 1973) the glandular cells stained positively for keratin and also for S-S and SH- groups. Some ultrastructural information was provided for the green turtle, Chelonia mydas (Aitken and Solomon, 1976). Glandular cells contained variable numbers of uniformly electron-dense, membrane-bound secretory granules. The luminal surface of gland cells sometimes had microvilli or exhibited blebbing. Golgi apparatus and RER were prominent. In the oviparous gecko Hemidactylus turcicus (which produces a hard, calcareous eggshell), the uterine gland cells contained loosely packed secretory granules, whereas in oviparous Saltuarius wyberba (which is thought to produce a soft, parchment-like eggshell) the granules were tightly packed (Chapter 3). The secretory granules in the few glands present in the viviparous gecko Hoplodactylus maculatus were smaller and more electron dense than those in either H. turcicus or S. wyberba (Chapters 2 and 3).

Vagina

The most posterior region of the oviduct, which leads out to the common urogenital/cloacal opening, is called the vagina (Botte, 1973; Palmer and Guillette, 1988; Picariello et al., 1989; Abrams Motz and Callard, 1991; Palmer et al., 1993; Perkins and Palmer, 1996; Chapters 2 and 3). The vagina is a thick, muscular region which acts as a sphincter during gravidity/gestation. The mucosa is thrown into deep folds which effectively reduce the volume of the vaginal lumen. The folds may reduce in height more posteriorly (Botte, 1973; Chapters 2 and 3) or, as in Sceloporus woodi, the trend is reversed and folds increase in height more posteriorly (Palmer et al., 1993). O. Cuellar (1966) suggested the folds may be needed to increase the mucosal surface area during oviposition/parturition.

The mucosal epithelium is heavily ciliated. Non-ciliated cells, which stain positively for carbohydrate substances, contain numerous secretory granules
(Chapters 2 and 3). In *Hoplodactylus maculatus*, goblet cells were also present and were filled with secretory granules which appeared to coalesce (Chapter 3). The vagina is usually aglandular, although glands, or gland-like crypts between the mucosal folds, may be present in the anterior vagina of some species and are associated with sperm storage (discussed below). There is a thick inner layer of circular muscle and a more compact outer layer of longitudinal muscle bounded by the serosa.

**HORMONAL CONTROL OF SEASONAL OVIDUCTAL DEVELOPMENT**

As with other reproductive organs, the oviduct undergoes changes over the course of a reproductive cycle. These changes can be correlated with seasonal hormone patterns. Hypertrophy of the oviduct occurs during the vitellogenic or pre-ovulatory period in preparation for gravidity/gestation and it is generally associated with an increase in the height and secretory nature of epithelial tissues. Mucosal glands and the muscularis also become hypertrophied at this time, and there may be a general increase in vascularity.

Seasonal changes in oviductal tissues have been reported in numerous reptilian species (for example: *Cnemidophorus inornatus* and *C. neomexicanus*, Christainsen, 1973; *Lacerta vivipara*, Gavaud, 1986; *Uromastix hardwickii*, Nawaz, 1987; *Hoplodactylus maculatus*, Chapter 3). Seasonal changes may simply be noted as changes in the weight of oviductal tissue, with predictably, maximum weight occurring during the period of reproductive activity (vitellogenesis and gravidity/gestation; Botte, 1973; van Wyk, 1984; Picariello et al., 1989; van Wyk, 1994). Similarly, oviductal weight increases significantly with sexual maturity (Sen and Maiti, 1990). Another common measure of oviductal hypertrophy is an increase in oviductal diameter and/or diameter of uterine glands such as that seen during the reproductive cycle of *Calotes versicolor* (Shanthakumari et al., 1992). In *H. maculatus*, epithelial cell height reached a maximum in late vitellogenic females before declining in pregnant females. In addition, the percentage of ciliated cells making up the epithelium changed significantly over the reproductive cycle with a maximum percentage occurring during gestation.

In the case of the turtle *Chrysemys picta*, the tubulo-alveolar glands of the uterine tube and the uterus showed significant growth in the pre-ovulatory period.
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(Abrams Motz and Callard, 1991). This occurred concurrently with maximal levels of both estradiol and progesterone (I. Callard et al., 1978). The thickness of oviductal tissues was maintained in post-ovulatory animals whilst progesterone levels were high, although glandular contents and granules from epithelial cells were discharged from the uterine tube. After oviposition, the uterine tube and uterus showed a significant regression concurrent with a drop to basal levels of estradiol and progesterone (I. Callard et al., 1978; Abrams Motz and Callard, 1991).

In the tortoise Gopherus polyphemus, epithelial cell height and the thickness of the mucosa increased in the uterine tube and the uterus during vitellogenesis (Palmer and Guillette, 1990). During gravidity, the epithelium remained hypertrophied, but the thickness of the uterine mucosa declined. Oviductal hypertrophy during vitellogenesis corresponded with elevated plasma estradiol concentrations. During gravidity, plasma progesterone concentration peaked.

A particularly detailed example of a reproductive cycle is available for the soft-shelled turtle, Lissemys p. punctata (Sarker et al., 1995; Sarker et al., 1996). Ovarian activity was divided into several different phases: preparatory, recrudescent, breeding, regressive and quiescent. The breeding phase was further divided into pre-ovulatory, ovulatory, post-ovulatory and post-laying stages. Plasma estradiol concentrations rose slowly through the preparatory and recrudescent phases, were high in pre-ovulatory females and reached their peak in ovulatory females (Sarker et al., 1996). Plasma estradiol concentrations declined sharply following ovulation, declined further in the post-oviposition phase, and were basal in regressive and quiescent phases. Progesterone concentrations remained low throughout the reproductive cycle, except in the breeding phase. Progesterone concentrations were low in the pre-ovulatory stage, increased during the ovulatory stage, reached their peak in the post-ovulatory phase and declined again following oviposition (Sarker et al., 1996). Absolute and relative (to body weight) oviductal weights (without eggs) were also considered in relation to these reproductive phases (Sarker et al., 1996). Weights remained low in the preparatory phase. They gradually increased during the recrudescent phase and peaked in the post-ovulatory stage of the breeding phase. Following oviposition, weights decreased sharply and remained low throughout the regressive and quiescent phases. These reductions in weight were associated with reductions in epithelial cell height, glandular size and secretory activity in both epithelial and glandular tissues (Sarker et al., 1995).
In the tropics, lizards are not characterised by a restricted breeding season. An
example is *Anolis pulchellus*, which was also interesting because it lays only a
single egg per clutch with alternating ovulations from each ovary (Ortiz and
Morales, 1974). The oviducts underwent cyclic glandular degeneration and
regeneration of new glands. This cycle was accelerated whilst an egg was in the
oviduct. As secretion occurred, glands broke down quickly, but at the same time
growth of new glands was occurring (Ortiz and Morales, 1974). A comparable
process of glandular degeneration and partial development of new glands was
seen in oviductal tissue cultured *in vitro*. These processes occurred more quickly
in oviductal tissue cultured with ovarian tissue than without ovarian tissue
(Ortiz and Morales, 1974).

I have included the above examples to illustrate differences in oviductal structure
and plasma hormone levels which occur over reproductive cycles. These
differences add to the complexity when considering which, and how, hormones
control oviductal function. Variability between individual species is always a
factor, and may cause problems to researchers searching for a common pattern of
control.

Control of oviductal development is a complex affair involving both direct and
indirect input from components of the hypothalamo - hypophysial - ovarian and
adrenal axes. Administration of exogenous hormones to non-reproductive, or to
surgically manipulated females, has been used on various occasions to analyse
the actions of hormones within oviductal tissue. To date, no one has
administered gonadotropin-releasing hormone (GnRH) to reptiles to analyse the
potential effects on oviductal tissue; in fact, there is very little information
concerning GnRH in reptiles at all (Licht and Porter, 1987). Some attention,
however, has been paid to the actions of gonadotropins on oviductal function.
In crocodilians and turtles, two gonadotropins similar to mammalian follicle-
stimulating hormone (FSH) and luteinising hormone (LH) have been identified.
whereas in squamates there appears to be only one gonadotropin with uncertain
homologies with mammalian FSH and LH (Licht, 1983). Little is known of the
seasonal fluctuations of gonadotropins in reptiles, although in the cobra *Naja
naja*, a bimodal profile of gonadotropin was observed with peaks during mid-
winter and vitellogenesis (Bona-Gallo et al., 1980).

In sexually quiescent *Lacerta sicula* (Botte and Basile, 1974), and early vitellogenic
*Sceloporus cyanogenys* (Callard et al., 1972a) and *Chrysemys picta* (Klicka and
Mahmoud, 1977), ovarian activity and oviductal development were stimulated after administration of pregnant mare serum or PMS (contains equine placental gonadotropin). In ovariectomised female *L. sicula*, administration of PMS caused no morphological changes in oviductal tissues, but there was an increase in DNA and protein content, and an increase in alkaline phosphatase activity (Botte and Basile, 1974). In post-partum *S. cyanogenys*, no effects of PMS were observed (Callard et al., 1972a). FSH, or FSH and LH, administered to ovariectomised *Hemidactylus flaviviridis* caused an increase in epithelial cell height and high activity of the enzymes Δ5-3β-HSD and G-6-PP (Haider, 1985). These changes were not noted in females treated with LH only. These results suggest gonadotropins may have direct or indirect effects on oviductal tissue without interaction with ovarian hormones such as estradiol.

The evidence to date suggests that estradiol, secreted by the ovary during vitellogenesis, is the main influence controlling oviductal development in preparation for gravidity/pregnancy. The oviductal development seen during vitellogenesis can often be mimicked in sexually quiescent or juvenile females treated with exogenous estradiol. Administration of estradiol typically causes oviductal hypertrophy with an increase in the number and size of mucosal glands, increased height and secretory activity in luminal and glandular epithelium, and increased oviductal vascularity (Prasad and Sanyal, 1969; Christainsen, 1973; Veith, 1974; Abrams Motz and Callard, 1991). Similarly, in ovariectomised females treated with exogenous estradiol, development of the oviduct is usually observed (Yaron, 1972; Mead et al., 1981; Chapter 4). The initial ovariectomy causes regression of oviductal tissues including a reduction in glandular activity and a reduction in epithelial cell height and secretory activity (Callard et al., 1972a; Mead et al., 1981; Haider, 1985; Chapter 4). Administration of exogenous estradiol will then cause oviductal hypertrophy. For instance, in the gecko *Hemidactylus turcicus*, the uterine epithelial layer of ovariectomised females was low and cuboidal with minimal/no differentiation or secretory activity (Chapter 4). Treatment with exogenous estradiol over a two week period resulted in a significant increase in cell height associated with gradual differentiation of the epithelium into ciliated and non-ciliated cells. Development of non-ciliated cells included production of secretory granules (low electron density) at the apical cell surface. Developing ciliated cells could be determined by the presence of cilia no taller than adjacent microvilli. The uterine shell glands showed less obvious changes over the course of treatment. Shell glands contained two cell types: dark cells with darkly staining nuclei and
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organelles, and light cells with very indistinct nuclei and organelles, except for prominent rough endoplasmic reticulum and free ribosomes. The differences between these two cell types were less distinct after two weeks of estradiol treatment, compared with glands at earlier stages of treatment, and glands then resembled those seen in naturally vitellogenic females (Chapter 4).

Although oviductal development was noted in ovariectomised *Thamnophis elegans* after estradiol treatment, this was only partial development in comparison to naturally vitellogenic females (Mead et al., 1981). This finding suggests that factors other than estradiol are also important in oviductal development. Additionally, the classical view of estradiol-supported oviductal development does not hold true in every species. In *Hoplodactylus maculatus*, no regression of oviductal tissue was observed following ovariectomy (Chapter 5). The oviducts in ovariectomised females resembled those in naturally vitellogenic females. The reasons for this unexpected result are unknown, but are hypothesised to involve plasma androgens.

Plasma concentrations of testosterone have been measured over the reproductive cycle in females of a few reptilian species. A peak of testosterone has been noted just prior to ovulation in some reptilian species (for example: I. Callard et al., 1978; Whittier et al., 1987; Guillette et al., 1997), whereas in others, testosterone was low or non-detectable over the entire reproductive cycle (Moore et al., 1985; van Wyk, 1994). As yet, little attention has been paid to the reasons for the different patterns of plasma testosterone observed in female reptiles.

Oviductal development has been induced by various androgens in several reptilian species. In the lizards *Cnemidophorus inornatus* and *C. neomexicanus*, a small amount of oviductal hypertrophy was induced with testosterone or testosterone propionate (Christainsen, 1973). In the gecko *Hemidactylus flaviviridis*, slight (but significant) oviductal hypertrophy was induced by estradiol; however, a greater increase in oviductal weight was noted after treatment with testosterone propionate, methyl testosterone, or 19-nortestosterone (Prasad and Sanyal, 1969). Testosterone also stimulated some oviductal hypertrophy in the spiny tailed lizard, *Uromastix hardwickii* (Akhtar, 1988), but not to the same extent as estradiol. As testosterone is a precursor for estradiol synthesis, it may be that localised conversion of plasma testosterone to estradiol by the oviduct may stimulate oviductal development (Guillette et al., 1997).
In the garter snake, *Thamnophis sirtalis*, females emerged from hibernation in the spring with low plasma concentrations of sex steroids (Whittier *et al.*, 1987). After mating, plasma estradiol concentrations increased rapidly over a 24 h period and then declined over the next two weeks. Thus, estradiol levels dropped several weeks before ovulation. This means that vitellogenesis and oviductal development were maintained when estradiol concentrations were at their lowest. Testosterone, however, increased just before ovulation, but only in those females which ovulated. This raises a question about the role that testosterone plays in oviductal growth. Intact snakes were treated with estradiol, testosterone (aromatisable), or 5α-dihydrotestosterone (DHT, non-aromatisable; Whittier, 1992). Females in all treatment groups exhibited an increase in oviductal mass with an associated increase in epithelial cell height and area in comparison to control females. DHT also drastically altered the oviductal morphology in comparison to other groups, with an increase in the number of pockets and blind-folding in the mucosal wall, suggesting that DHT is unlikely to play a role in normal oviductal development. An interesting point is that no androgen receptors have been detected in oviductal tissues of *T. sirtalis* (Whittier *et al.*, 1991), suggesting that the effects of androgens on oviductal tissues are not mediated by an androgen receptor mechanism in this species. However, androgen receptor and aromatase activity have been identified in the oviduct of the turtle *Trachemys scripta* (S. Smith *et al.*, 1995).

Another sex steroid hormone of interest is progesterone. Progesterone is secreted by the corpus luteum (Veith, 1974; Arslan *et al.*, 1978) and has been implicated in several oviductal functions. Of particular interest is the role that progesterone plays maintaining gestation (discussed below). Progesterone may also act during oviductal growth, but research to date does not provide consistent information. In the viviparous lizard, *Xantusia vigilis*, there was no significant difference in oviductal weight between ovariectomised females treated with vehicle solution and those treated with progesterone (Yaron, 1972). An increase in oviductal weight was seen in ovariectomised females treated with estradiol, and this increase was not as great in females treated with both estradiol and progesterone. A similar pattern was seen in *Thamnophis elegans*, except that progesterone administered concurrently with estradiol produced effects no different from those of estradiol alone (Mead *et al.*, 1981). In the lizard, *Sceloporus cyanogenys*, progesterone administered concurrently with PMS prevented the oviductal development seen in females treated with PMS only (Callard *et al.*, 1972a).
Prolactin (a pituitary hormone) is yet another hormone which has been considered briefly in relation to oviductal activity. In *Anolis carolinensis*, injections of ovine prolactin depressed oviductal weights in vitellogenic females, but not in sexually quiescent females (Hensgen et al., 1980). Injection of prolactin did not inhibit oviductal development in females treated with exogenous estradiol. However, prolactin did diminish the ability of ovine FSH to stimulate oviductal growth in vitellogenic females (Hensgen et al., 1980). Hensgen et al. (1980) suggested that these results indicated that prolactin acts on the ovary by suppressing growth and steroid biosynthesis of smaller ovarian follicles and that reproductive status can influence prolactin effects. Possible diurnal and seasonal fluctuations in prolactin have not been investigated as a prolactin assay has not been developed for reptilian species.

In summary, oviductal tissue shows seasonal changes which correlate with seasonal hormone cycles. Estradiol, which has its highest plasma concentrations during vitellogenesis, appears to be the main influence controlling the development of the oviduct in preparation for gravidity/gestation. The hypertrophy of the oviduct seen in vitellogenic females can be mimicked in sexually quiescent females or ovariectomised females if exogenous estradiol is administered. Estradiol does not act in isolation, however, and other hormones (gonadotropins, androgens, progesterone, prolactin) may influence oviductal development. Other hormonal and neural influences act to cause parturition/oviposition and these will be discussed later.

**Sex Steroid Receptors**

That sex steroids act directly on oviductal tissue is implied by the presence of sex steroid receptor proteins. Estrogen receptors (ER) have been identified in the oviduct of several reptilian species (turtles: *Chrysemys picta*, Salhanick et al., 1979; Giannoukos and Callard, 1996; squamates: viviparous snake *Thamnophis sirtalis parietalis*, Whittier et al., 1987; oviparous lizard *Podarcis s. sicula*, Paolucci et al., 1992; Paolucci and DiFiore, 1994; crocodilians: *Alligator mississippiensis*, Vonier et al., 1997). Ovariectomy significantly decreased ER concentration in *C. picta* (Giannoukos and Callard, 1996) and *P. s. sicula*. In ovariectomised *P. s. sicula*, estradiol treatment increased ER concentration and induced an ER shift from the cytosol into the nuclei (Paolucci et al., 1992). ER levels in ovariectomised females were not restored by any steroid regime in *C. picta* (Giannoukos and Callard, 1996).
ER quantity may change over a reproductive cycle. In _P. s. sicula_, ER concentration significantly increased as the oviduct developed, supporting a role for estradiol in oviductal stimulation (Paolucci _et al._, 1992). Ovariectomised or post-ovulatory females had little oviductal ER-mRNA in comparison to estrogen treated or vitellogenic female _Cnemidophorus uniparens_ (Young _et al._, 1995).

Progesterone receptors (PR) have also been identified in reptilian oviductal tissue (squamates: viviparous snake, _Neroida_, Kleis-San Francisco and Callard, 1986; oviparous lizard _Podarcis s. sicula_, Paolucci and DiFiore, 1994; turtles: _Chelydra serpenta_, Mahmoud _et al._, 1986; _Chrysemys picta_, Ho and Callard, 1984; Giannoukos and Callard, 1996; crocodilians: _Alligator mississippiensis_, Vonier _et al._, 1997). It appears there are two isoforms, PR-A and PR-B (Reese and Callard, 1989; Paolucci and DiFiore, 1994). PR-B, but not PR-A, is under estrogenic control and shows seasonal changes. Immunocytochemical analysis identified PR in the nuclei of uterine epithelium, submucosal glands, and smooth muscle in _C. picta_ (Giannoukos and Callard, 1996).

Numbers of PRs change over a reproductive cycle (Ho and Callard, 1984). In the snapping turtle, _Chelydra serpentina_, no PRs were detected during the post-ovulatory phase (when plasma progesterone concentrations were high and the corpora lutea were active; Mahmoud _et al._, 1986). Estrogen priming did not increase PRs at this time. In the post-oviposition phase, PR were only detectable after estrogen priming. During vitellogenesis, however, PR were detectable without estrogen priming. Both cytosolic and nuclear PRs increased significantly during vitellogenesis in the viviparous snake _Nerodia_ (Kleis-San Francisco and Callard, 1986). Levels of cytosolic PR then declined, but nuclear PR did not decline until the second trimester of gestation. In unisexual _Cnemidophorus uniparens_, ovariectomised or post-ovulatory females had little oviductal PR-mRNA in comparison to estrogen treated or vitellogenic females (Young _et al._, 1995).

Treatment or priming with estradiol up-regulated PR in _T. scripta_ (Selcer and Leavitt, 1991), _P. s. sicula_ (Paolucci and DiFiore, 1994) and _Nerodia_ (Kleis-San Francisco and Callard, 1986), but PR was down-regulated if progesterone was administered with or after estradiol treatment. Progesterone administered alone had no effect on PR number in _T. scripta_ (Selcer and Leavitt, 1991) or _Nerodia_ (Kleis-San Francisco and Callard, 1986), but appeared to promote redistribution of PR to the nuclear sites in _Nerodia_. In the turtle _C. picta_ also, progesterone down
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regulated PR in both intact and ovariectomised females, but estradiol did not elevate PR concentration above controls (Giannoukos and Callard, 1996). Ho and Callard (1984) hypothesised that PR may be maintained by estrogens and translocated from the cytosol to nuclear sites by progesterone. In *Podarcis s. sicula*, the peak of ER in nuclei preceded an increase in occupied PR which suggested a sequential action of estradiol and progesterone in the oviduct (Paolucci and DiFiore, 1994).

Androgen receptors have been identified in the oviduct of the turtle *Trachemys scripta* (S. Smith *et al.*, 1995). Seasonal changes in the number of androgen receptors, or potential changes following hormonal manipulation, have not been investigated.

In summary, estrogen and progesterone receptors have been identified in representative species of turtle, squamate and crocodilian. Concentrations of these receptors vary over a reproductive cycle and may be influenced by surgical and hormonal manipulation. Thus, receptivity of oviductal tissues to various hormones will change depending on the interaction between changing hormone and hormone-receptor levels. Androgen receptors have been detected in one species of reptile, however, further information concerning changes over a reproductive cycle and following experimental manipulation will be useful.

*Enzyme Activity*

In addition to steroid hormone receptors, certain key enzymes involved in the biosynthesis of steroid hormones have been identified in the oviduct of several reptilian species. For instance, activity of Δ⁵-3β-hydroxysteroid dehydrogenase (HSD; involved in the conversion of pregnenolone to progesterone) and 17β-HSD (involved in oxidative inter-conversions of various estrogenic and androgenic steroids) was measured in the uterine glands of the skink *Mabuya carinata* (Mundkur and Sarkar, 1982) and in the mucosal epithelium of the agamid *Calotes versicolor* (Shanthakumari *et al.*, 1990, 1992). Various other metabolic enzymes were also detected in the uterine glands of *M. carinata* (glucose-6-phosphate dehydrogenase, NADH₂ diaphorase, and lactate dehydrogenase; Mundkur and Sarkar, 1982).

Most of the steroid and metabolic enzymes measured in *C. versicolor* showed the highest activities during the reproductive phase and a decrease following
breeding (Shanthakumari et al., 1992). B-glucuronidase was an exception, with highest activity occurring in the regressed oviduct. Enzymatic activities showed some variation among the different regions of the oviduct. For example, acid phosphatase activity was highest in the uterine regions as compared to the infundibulum and vagina. Shanthakumari et al., (1992) hypothesised that the presence of these enzymes means the oviduct is a site of steroid metabolism and the seasonal changes in these enzymes suggests they may be under the control of estrogenic hormones. In the case of B-glucuronidase, the inverse activity may be related to tissue degeneration following breeding as it is known to be a lysosomal enzyme (Shanthakumari et al., 1992).

A similar suite of enzymes (see above, Shanthakumari et al., 1992) to those measured in the mucosal epithelium was also detected in the epithelium of uterovaginal sperm pockets of C. versicolor (Shanthakumari et al., 1990) and Psammophilus dorsalis (Srinivas et al., 1995). Whether these enzymes have a role in sperm storage is unknown.

Shanthakumari et al. (1992) suggested acid phosphatase provided the best indicator of secretory activity in uterine glands. Alkaline and acid phosphatase activity were also noted in the oviducts of Lacerta sicula during the pre-ovulatory and ovulatory phase (Botte, 1973). Activity was noted in the epithelium of the infundibulum and uterine tube. In the uterus, activity was noted in shell glands. Along the entire oviduct, the walls of blood vessels are rich in phosphatase activity. Botte (1973) hypothesised that the presence of phosphatase activity indicated that these enzymes are involved with secretion and also in the exchange between blood vessels and the mucosa.

In summary, numerous steroidogenic and metabolic enzymes have been identified in the reptilian oviduct, the activities of which change over a reproductive cycle. These findings indicate that the oviduct is a site of steroid metabolism.

**Growth Factors**

In recent years, numerous growth factors and cytokines have been identified in mammalian species. These substances act in an autocrine, paracrine and endocrine manner to produce a response. The continuing work to identify these polypeptides, their binding proteins and receptors, and to determine their
function and mechanism of action, has resulted in a vast and initially bewildering body of literature. These growth factors obviously play a vital role in reproduction, including the actions of the oviduct, as well as in other bodily functions.

Research on growth factors and cytokines in reptilian species has only just begun. The growth factor which has received the most attention to date is insulin-like growth factor (IGF). Both IGF-I and II were identified in skeletal tissue of a gecko (Bautista et al., 1990). IGF-I has been detected in the plasma of the alligator *Alligator mississippiensis* and the turtle *Trachemys scripta* (Crain et al., 1990). Maximal plasma levels of IGF-I were observed during gravidity in *A. mississippiensis*, correlating with high plasma progesterone concentrations (Guillette et al., 1996). It was hypothesised that high levels of IGF-I in gravid females may be due to the synthesis of IGF-I for incorporation into eggs and that IGF-I may also act to stimulate uterine secretion. Both IGF-I and II have been found in the albumen of fully shelled eggs *in utero* from *A. mississippiensis* (Guillette and Williams, 1991). IGF-I immunoreactivity was noted in the mucosal glands of the uterine tube and uterus, and in the uterine luminal epithelium and its secretions, in the alligator *A. mississippiensis* and the tortoise *Gopherus polyphemus* (Palmer and Guillette, 1991; Cox and Guillette, 1993). In mammals, IGF-I has been implicated as a possible mediator of estradiol-induced proliferation of the female reproductive tract, as well as having involvement in placental interactions and embryonic development (Simmen and Simmen, 1991; Wang and Chard, 1992).

Epidermal growth factor (EGF) is also believed to act as a facilitator of estrogen action and causes proliferation of mouse, rabbit and human endometrial cells (S. Smith, 1994). EGF provides an interesting example of the complex interactions that occur in oviductal tissue. In mice, estradiol stimulated uterine and vaginal growth *in vivo*. However, to elicit the epithelial differentiation and hypertrophy *in vitro*, epithelial tissue had to be cultured in combination with stromal tissue (Cooke et al., 1986), suggesting paracrine growth factors were involved. Further evidence supporting this hypothesis was provided by Nelson et al. (1991) who showed that EGF, administered to ovariectomised mice, acted as a potent uterine and vaginal mitogen. Additionally, an antibody specific for EGF significantly inhibited estrogen-induced uterine and vaginal growth (Nelson et al., 1991). In human endometrial gland preparations, but not stromal cell preparations, EGF increased mean cell counts (Haining et al., 1991). EGF derived from the
endometrium has also been suggested to facilitate embryonic development (S. Smith, 1994). In studies concerning reptiles, EGF immunoreactivity has been identified in the mucosal glands and the luminal epithelium of the uterus in the alligator *Alligator mississippiensis* (Palmer and Guillette, 1991).

In the Mediterranean gecko, *Hemidactylus turcicus*, both exogenous IGF-I and EGF induced hypertrophy of the oviduct in ovariectomised females (Cox, 1994). Epithelial cell height was significantly increased in comparison to control females, but only 25% of the height measured in estradiol treated females. In IGF-I treated females, mucosal thickness and gland diameter were 71% and 83% respectively of that observed in estradiol treated females. In EGF treated females, mucosal thickness and gland diameter were only 50% and 65% of that recorded in estradiol treated females. The major difference between treatments appeared to be in the number and density of secretory granules produced. These results suggest that IGF-I and EGF can cause oviductal development (but to a lesser extent than estradiol) and that IGF-I and/or EGF do not act in isolation within the oviduct.

Interleukin-I (IL-I) has also been found in reptiles. IL-I, and its specific membrane receptor (IL-1R tl), have been identified using immunocytochemistry in the oviductal epithelium of *Chalcides chalcides* during pre-pregnancy, early gestation and post-partum (Paulesu *et al.*, 1995). Immunoreactivity was much higher in the pregnant, as compared with the pre- and post-pregnant, uterus. IL-1α and IL-1β immunoreactivity was observed in the placenta of *C. chalcides* during mid-gestation, particularly at the basal region of cells. Immunoreactivity was noted in uterine epithelium, but only in a small number of cells; however, there was clear immunoreactivity in uterine connective tissue. Just prior to parturition, the epithelium showed strong staining, particularly around the nuclei and at the apical portion of cells where apocrine secretion appeared to be occurring. Although the technique used cannot differentiate between absorbed and secreted IL-I, the results to date suggest that IL-I may be involved in placentation. In mammals, it has been suggested, amongst other actions, that IL-1 inhibits the proliferation of stromal and epithelial endometrial cells, is pro-inflammatory and induces production of prostaglandin-E₂ (see Tabibzadeh, 1994 for summary).

As well as EGF, IGF-I and -II, and interleukins (considered above), the list of growth factors and cytokines identified in mammals includes (amongst others) transforming growth factor-α and β, fibroblast growth factors and colony
stimulating factors. It is an inappropriate forum here to discuss all the possible oviductal interactions of growth factors and cytokines which have been identified in mammalian species. Reviews which summarise the current information are available (for example: Simmen and Simmen, 1991; Murphy and Barron, 1993; S. Smith, 1994; Tabibzadeh, 1994). It is appropriate, however, to identify how these peptides cause effects in the reproductive tract of mammals and where continued study in reptiles will be invaluable to the study of oviductal action. Growth factors are involved in the development of the oviduct in association with the hypothalamo-pituitary axis, acting in a mitogenic and angiogenic fashion. It appears that this development is differentially regulated; a whole suite of factors may be necessary for complete development and regression. Production of oviductal secretions (mucus, albumen, shell membranes) and their release at the appropriate time may well involve growth factors. Maternal growth factors, synthesised by the oviduct or transported via the oviduct, may be essential for complete embryonic development. Growth factors of foetal origin may be involved in regional differentiation of the oviduct associated with placentation and may also be necessary to maintain gestation and later to trigger parturition.

In summary, EGF, IGF-I and -II, and IL-I have been identified in the reptilian oviduct. EGF and IGF are believed to act as facilitators of estradiol-induced proliferation. IL-I is believed to act in placentation. There are numerous areas where the above growth factors, and others yet to be identified in reptiles, may be important in the control of various oviductal functions. It will be necessary to characterise the chemical properties of these growth factors, their receptors and binding proteins, before their exact function(s) can be determined.

FUNCTIONS OF THE OVIDUCT

Functions of the luminal epithelium

In all regions of the oviduct, the luminal epithelium has secretory potential. The secretions include albumen, components of the eggshell and materials involved with placentation and sperm storage. These will be discussed more fully below. There are three cell types found in the luminal epithelium: ciliated, microvillous non-ciliated and bleb-like non-ciliated cells. The function of ciliated cells is presumably to maintain movement of mucus and cellular debris down the oviduct (Palmer and Guillette, 1988). Cilia may aid in the movement of sperm
and potentially the ovulated egg also. Apical protrusions and blebbing from ciliated cells have been noted (Chapter 2), suggesting that ciliated cells may also have some secretory function.

Microvillous non-ciliated cells are presumably mucus-producing, mucus being necessary to keep the lumen of the oviduct moist and clean (Aitken and Solomon, 1976; Leese, 1988). The oviductal lumen is continuous with the animal's exterior (via the urogenital sinus) and, as such, is vulnerable to contamination. Whether oviductal secretions contain anti-bacterial, anti-fungal or anti-viral properties has not yet been investigated in reptiles.

Bleb-like non-ciliated cells have been identified only in a small number of species (Palmer and Guillette, 1988; Chapters 2 and 3) and their function is unknown. Palmer and Guillette (1988) suggested they may be involved in apocrine or merocrine secretion.

**Sperm Storage and Fertilisation**

Sperm storage has been noted in many reptilian species, and reviewers such as Birkhead and Moller (1993) have considered the evolutionary implications of such storage. Sperm storage is considered obligatory in some species due to asynchronous reproductive cycles in males and females. It allows copulation to be separated from fertilisation. Sperm storage may also be advantageous as it extends the reproductive period available to females, it may contribute to sperm competition and/or multiple paternity within a single clutch (Birkhead and Mollar, 1993; Gist and Fischer, 1993), it may reduce the risk of predation by reducing copulation frequency (Conner and Crews, 1980), and it may act as insurance against not finding a partner due to low densities or slow movement of any one species (Birkhead and Mollar, 1993).

Sites of sperm storage appear to be restricted to either the anterior vagina and/or the anterior oviductal regions (usually the posterior uterine tube). For instance, in the oviparous snake, *Diadophis punctatus*, two sites for sperm storage were noted (Perkins and Palmer, 1996). After mating in either the autumn or spring, sperm were stored in folds of the mucosa in the anterior vagina. During the early stages of vitellogenesis, sperm travelled up through the uterine lumen to glands in the posterior uterine tube. A similar pattern was observed in the viviparous gecko, *Hoplodactylus maculatus* (Chapter 2).
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Sperm storage in the anterior vagina has been reported for several reptilian species (O. Cuellar, 1966; Halpert et al., 1982; Shanthakumari et al., 1990; Palmer et al., 1993). Sites for sperm storage are often formed from crypts between folds of the vaginal mucosa (Psammophilus dorsalis, Srinivas et al., 1995). In Anolis carolinensis, sperm storage occurred in tubular outgrowths of the anterior vagina (W. Fox, 1963; Conner and Crews, 1980).

Storage in the posterior uterine tube is also common. This site of storage has been identified in several gekkonid species (for example: Picariello et al., 1989; Murphy-Walker and Haley, 1996; Chapter 2). Sperm storage was also noted in the infundibulum (including uterine tube) of the geckos Phylloactylus homolepiturus and Coleonyx variegatus (O. Cuellar, 1966) and the geckos belonging to the Heteronotia binoei complex (Whittier et al., 1994). Gist and Jones (1989) identified sperm storage tubules, which communicated with the oviductal lumen via ducts, in the posterior uterine tube of 11 species of turtle representing six families.

To my knowledge, the only ultrastructural information concerning sperm storage sites is for the box turtle, Terrapene carolina (Gist and Fischer, 1993). In this species, sperm storage sites were identified in the posterior uterine tube (Hattan and Gist, 1975; Gist and Fischer, 1993). The tubules containing sperm, which were no different from others in the uterine tube, were surrounded by six-eight secretory cells which contained numerous membrane bound vesicles (Gist and Fischer, 1993). Microvilli were present on the apical membranes and prominent junctional complexes were present on the lateral membranes. Sperm were not in contact with the oviductal tissues. That the sperm-carrying and non-sperm-carrying tubules did not differ suggested that their function as sperm storage structures may be fortuitous. Gist and Jones (1987) pointed out that sperm storage structures are characteristically unspecialised except, of course, for the presence of sperm.

Although the morphology of sperm storage structures may not differ from that of the surrounding oviduct, biochemical and physiological differences may play a role in sperm maintenance. In the majority of papers available, sperm are described as being in groups in the sperm storage sites, without direct connection to the oviductal tissue (W. Fox, 1963; Gist and Jones, 1989; Shanthakumari et al., 1990; Gist and Fischer, 1993). However, in Holbrookia propinqua the sperm were considered to be directly associated or partially embedded in oviductal tissue.
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(Adams and Cooper, 1988). This suggests that in the majority of cases, either the oviduct must secrete, or the male semen must supply, any substances associated with sperm maintenance.

In the rock lizard, *Psammophilus dorsalis*, discrete granules resembling those found in the male's vas deferens were found associated with sperm in the anterior vagina (Srinivas et al., 1995). These granules stained positively for carbohydrate (PAS) and also for the enzyme acid phosphatase. In the agamid lizard *Calotes versicolor*, sperm were mixed with a PAS-positive homogeneous mixture, although it is not known whether this was of oviductal origin, or from the male (Shanthakumari et al., 1990). The sperm in *C. versicolor* were stored in pockets in the uterovaginal region. The epithelium of these pockets resembled that of the oviductal epithelium and stained positively for proteins and carbohydrates. Activity of various steroidogenic and metabolic enzymes was also detected in the epithelium (Shanthakumari et al., 1990).

In the red-sided garter snake, *Thamnophis sirtalis parietalis*, six weeks into the period of winter dormancy the epithelial cells of the vagina (where sperm storage occurs) hypertrophied and stained strongly for carbohydrate (Halpert et al., 1982). The epithelial border sloughed off and was associated with the stored sperm as it moved anteriorly. After 20 weeks in dormancy, the sperm were found in storage tubules in the posterior uterine tube. Halpert et al. (1982) called this the carrier matrix and suggested it facilitated transport of the sperm anteriorly and may function as a nutritional store. This suggests an active role by the oviduct of this species in sperm transport and maintenance.

Another factor to consider is how sperm are transported within the oviduct, and how sperm are released from storage sites. In *Anolis carolinensis*, sperm entered the storage tubules (in the anterior vagina) 2-6 h after insemination (Conner and Crews, 1980). Small amounts of sperm also reached the uterine tube 6-24 h after mating. It was suggested by Halpert et al. (1982) that the direction of ciliary beating may be reversed during vitellogenesis to aid the movement of sperm up to the oviduct towards the infundibulum. Halpert et al. (1982) also hypothesised that ovulation may trigger the release of sperm from storage sites in the uterine tube. As the eggs pass though the uterine tube, the glands containing sperm are stretched which causes the release of sperm into the lumen. This idea, however, does not explain how sperm from vaginal sites are released.
That stored sperm are capable of fertilisation is suggested by the fact that females of several species which have been kept in captivity without males for prolonged periods can still produce viable clutches (for instance, Holbrookia propinquua, Adams and Cooper, 1988). As an extreme example, delayed fertilisation and the production of a fully-developed embryo occurred in the snake Acrochordas javanicus after seven years in captivity (Mangusson, 1979). However, H. Fox (1977) advised caution in assuming sperm storage from delayed fertilisation as parthenogenesis occurs in some species. In the gecko Hemidactylus frenatus, females maintained in isolation for a period of one year produced, on average, seven clutches suggesting a minimum period of sperm storage of 36 weeks (Murphy-Walker and Haley, 1996). Hatchlings included males which excluded the possibility of parthenogenesis causing the additional clutches.

The site of fertilisation in reptilian species has yet to be determined. Fertilisation must presumably occur before the ovulated oocyte is covered by albumen or shell membranes. Eggs are coated with oviductal secretions as soon as they enter the infundibular ostium (Palmer et al., 1993). This suggests fertilisation must occur in either the infundibulum or uterine tube and sperm have been observed in storage sites in both these regions. For instance, in a gecko from the Heteronotia binoei complex that was in the process of ovulation, nests of sperm were observed in the oviductal wall of the infundibulum that surrounded the unshelled ovum (Whittier et al., 1994). This suggests fertilisation occurs in the infundibulum of this species.

To summarise this section, although storage of sperm has been noted in several species, we are none the wiser as to the contribution the oviduct may have in providing for sperm maintenance, be it nutrition, protection or otherwise. It is possible also, that the oviduct does no more than provide a site for sperm storage. We do not know the mechanism by which sperm are released from storage sites in either the vagina or anterior oviductal regions, nor whether the oviduct plays a role in the movement of sperm up the oviduct to the site of fertilisation. The site of fertilisation is yet another unknown and research analysing the oviduct and egg at timed periods following ovulation would be appropriate.

*Albumen Production*

The eggs of turtles and crocodilians are covered with a thick layer of albumen (egg white proteins) at oviposition, whereas lepidosaurian eggs apparently lack such a
layer (M. Packard et al., 1988). Reptilian albumen, like that of birds, is believed to have various anti-microbial, nutritive, supporting, cushioning, and water binding properties essential for the developing embryo (Palmer and Guillette, 1991). It also provides an important reservoir of water, which in birds is secreted by the shell gland as 'plumping fluid'. M. Packard et al. (1988) suggested the lack of an albumen layer in lepidosaurians means that the eggs contain insufficient water when they are oviposited to support the embryos until they hatch. However, significant water intake by the egg in the oviduct of lepidosaurian Anolis pulchellus has been reported (Cordeno-López and Morales, 1995).

It is thought that the uterine tube is responsible for secretion of the albumen layer. The uterine tube of the turtle and crocodilian oviduct is homologous with the avian magnum which is known to secrete albumen (Aitken and Solomon, 1976). Using tritiated leucine and explant cultures, it was shown that the uterine tube of Pseudemys s. scripta is capable of synthesising and secreting albumen proteins in vitro (Palmer and Guillette, 1991). Additionally, antibodies to whole fowl albumen and purified ovalbumen showed cross-reactivity to reptilian albumen proteins and bound to mucosal glands in the uterine tube of Alligator mississippiensis (Palmer and Guillette, 1991).

Despite there being no albumen layer in lepidosaurians, various albumen proteins have been detected in the eggs of certain squamates (Diadophis punctatus, Sceloporus woodi, S. virgatus, S. scalaris, and Anolis sagrei; Palmer and Guillette, 1991). It is not known, however, whether these proteins are of oviductal or ovarian origin. Synthesis of avidin (a major avian albumen protein) was detected in the oviduct of Lacerta sicula (Botte et al., 1974; Botte and Granata, 1977). Estradiol, or estradiol and progesterone, stimulated avidin synthesis in the uterine tube. Testosterone also increased avidin synthesis, but was less potent. However, without a distinct albumen layer, the question arises as to whether and where these proteins are incorporated into the egg.

As in other lepidosaurians, there is no structural distinction between the yolk and albumen layer in Anolis pulchellus (Cordeno-López and Morales, 1995). Electrophoresis showed no qualitative difference between the follicle and the egg, suggesting that, at least in A. pulchellus, there are no proteins of oviductal origin in the egg (Cordeno-López and Morales, 1995).
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Thus, there is obviously still some contention surrounding the presence of albumen in squamate species. It appears that several considerations need to be addressed. Does the lack of a distinct albumen layer in lepidosaurians mean that proteins of oviductal origin are not found in the eggs? If oviductal proteins are detected, are they incorporated into the yolk layer or elsewhere? What is the function of these potential oviductal proteins in lepidosaurians? Do they share the same functions as those proposed for albumen in turtles and crocodilians?

Eggshell Production

A vital function of the reptilian oviduct in oviparous species is the production of the eggshell. The generalised reptilian eggshell is composed of an inorganic layer of calcium bicarbonate (either calcite or aragonite) with an underlying organic layer(s) which is otherwise known as the shell membrane (see M. Packard and DeMarco, 1991). The innermost layer of the shell membrane, which lies adjacent to the extra-embryonic membranes, is known as the inner boundary. Eggshell structure varies widely between species, with the different layers of the eggshell differing in thickness and morphology. The differences in eggshell morphology will presumably be reflected by differences in those oviductal regions responsible for secretion of the eggshell. Several useful and detailed reviews concerning reptilian eggshell structure are available (M. Packard et al., 1982; M. Packard and Hirsch, 1986; M. Packard et al., 1988; M. Packard and DeMarco, 1991).

The inner boundary is thought to be secreted by the anterior regions of the oviduct. It is known that oviducal secretions are present on the egg as soon as it enters the infundibular ostium (Palmer et al., 1993) and it has been hypothesised that the infundibulum is responsible for secretion of the inner boundary (Guillette et al., 1989). However, in those species with a layer of albumen present, which lies interior to the shell membrane, presumably the inner boundary must be secreted by the uterine tube, isthmus or uterus. Cree et al. (1996) hypothesised that the uterine glands are responsible for secretion of the inner boundary as well as the shell membrane of tuatara, based on similarities between secretory granules observed in the uterine glands of tuatara (and other reptiles) and those forming the inner boundary of the tuatara eggshell.

In turtles and lepidosaurians, the uterus is a single region (unlike the crocodilians in which the uterus is divided into two functionally different regions) which produces both the calcareous and fibrous components of the
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The fibres making up the shell membrane are secreted by the uterine mucosal glands. Once the egg reached the uterus of *Sceloporus woodi*, long, proteinaceous fibres were observed extruding from ducts of the mucosal glands (Palmer et al., 1993). Most glands had stopped secreting by 24 h post-ovulation and the shell membrane was largely complete. In *Lacerta sicula* also, secretory material in the form of filaments was noted passing into the lumen of the oviduct from the uterine mucosal glands (Botte, 1973). The fibres had the same histochemical properties as the secretion noted within the glands. Palmer et al. (1993) hypothesised that the formation of the fibrous membrane is similar to that seen in birds. As the proteinaceous material is forced out of the neck of the gland it coalesces into a fibre. It appears that the orientation of the fibres forming the shell membrane is due, at least in part, to the rotation of the eggs within the oviduct (M. Packard and DeMarco, 1991; Palmer et al., 1993).

In a study comparing two lizard species, the uterine mucosal glands of *Crotaphytus collaris* produced fibres of a collagen-like material, whereas in *Eumeces obsoletus*, the uterine mucosal glands did not stain for collagen (Guillette et al., 1989). This highlights the potential difference in chemical composition of the shell membrane between species. Guillette et al. (1989) commented that additional studies are required to determine if phylogenetically distinct reptilian species secrete fibres which differ biochemically and/or structurally.

The exact source of calcium needed for eggshell production is still unknown, although various clues suggest that the uterine epithelium is responsible. In *Lissemys p. punctata*, the secretory cells of the uterine epithelium stained positively for calcium throughout the period of gravidity (Sarker et al., 1995). In *Crotaphytus collaris* also, but not *Eumeces obsoletus*, the uterine epithelium stained intensely for calcium (Guillette et al., 1989). The uterine epithelium of *Sceloporus woodi* exhibited changes during gravidity which correlated with the period of calcium deposition (Palmer et al., 1993). During the period of calcium deposition, the apical membranes were greatly distended, the cells were hypertrophied and the microvilli were less pronounced. There is evidence, however, which does not support the uterine epithelium as the source of calcium. Picariello et al. (1989) used chlorotetracycline chloride (fluorescent) to monitor Ca\(^{2+}\) in the oviduct of the gecko *Tarentola m. mauritanica*. During the period of maximum reproductive activity fluorescence was noted in the basal region of uterine glands, but not in the epithelium. Calcium, however, is
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7.33

essential for mitochondrial function and, as mitochondria are common in both glandular and epithelial cells, the presence of calcium staining in various regions does not necessary correspond to calcium secretion for eggshell production.

The extant archosaurs (birds and crocodilians) exhibit similar egg-shelling processes. As with birds, different components of the crocodilian eggshell are secreted in different regions of the oviduct (Palmer and Guillette, 1992). In crocodilians, it appears that two spatially distinct regions, the anterior and posterior uterus, are responsible for the production of the shell membrane and the calcareous component respectively. The anterior uterus of *Alligator mississippiensis* resembled the isthmus of birds and the uterus of other reptiles (Palmer and Guillette, 1992). However, the mucosal glands of the posterior uterus in *A. mississippiensis* resembled the glands of the avian shell gland which is responsible for production of the calcareous component of the eggshell. The cells of the mucosal glands in the posterior uterus may function to secrete calcium ions as well as adding the 'plumping water' needed to saturate albumen proteins (Palmer and Guillette, 1992).

The structure of reptilian eggshells varies widely between species. Guillette and Jones (1985b) suggested that the differences in eggshell structure may relate to the structural organisation of the uterine shell glands. For instance, in *Sceloporus a. aeneus*, the shell glands had distinct pores which opened out onto the luminal surface. These pores, however, did not cover the entire surface of the epithelium. The particular arrangement of the pores may contribute to the irregular surface of the eggshell (Guillette and Jones, 1985b).

To summarise, little is known about the oviduct's role in the process of egg shelling in reptiles. Although the oviducal regions where shelling occurs have been determined, the details available concerning the process during shelling within these regions are minimal, particularly the details of calcium secretion. The hormonal mechanisms controlling eggshell production are unknown. H. Cuellar (1979) observed that deluteinisation disrupted the shelling process in the lizard *Cnemidophorus uniparens*. This suggests a possible role for the corpus luteum and its major hormonal product, progesterone, in the shelling process. Equally, the stress of the process of deluteinisation may have been the casual factor disrupting shelling. More detailed research determining the hormonal triggers for shelling is needed.
Placentation

A feature considered essential to the evolution of viviparity is the development of a placenta, which is defined as any intimate apposition or fusion of parental to foetal tissues allowing for physiological exchange (Mossman, 1937). In viviparous squamates, the extra-embryonic membranes (which are also present within the eggshell of oviparous species, Fig. 7.2) provide the foetal component of the placenta, whilst the uterus provides the parental component. The presence of an ovulated egg distends the uterus meaning that most of the egg surface is in contact with the uterus. Additionally, as the eggshell is reduced or absent in viviparous species, the uterine epithelium is very closely apposed to the extraembryonic membranes (Blackburn, 1993a). The thin shell membrane found in some viviparous species is believed to be due to the few uterine mucosal glands (responsible for secretion of the shell membrane in oviparous species) still present in viviparous species. In Hoplodactylus maculatus, the thin shell membrane was only observed during the early stages of pregnancy (Boyd, 1942; Chapter 2). The mechanism by which the shell membrane is lost during pregnancy is unknown.

Despite detailed morphological descriptions of placental structures in some species, which range from apposition of extraembryonic membranes with uterine tissue to complex hypertrophy and interdigitation, no detailed information concerning the function of placental tissues is available. There are detailed reviews of morphology and ontogeny of placental structure concerning the species studied to date (Yaron, 1985; Stewart and Blackburn, 1988; Blackburn, 1993a; Stewart, 1993) and further elaboration here would be redundant. Instead, consistent with the theme of this review, I will focus on the structure and function of the uterus in placentation.

The majority of viviparous squamates ovulate large, yolky eggs and have a conservative yolksac and chorioallantoic placenta. The uterus may well exhibit regional differences in structure associated with the different placental regions. Commonly, the uterus is very thin during pregnancy, particularly the epithelial layer which may be cuboidal or squamous. Numerous blood vessels are present at the base of the epithelium (Boyd, 1942; Stewart, 1992) allowing the maternal blood supply to be very close to the foetal blood supply.
Figure 7.2. Diagram illustrating extraembryonic membranes within A: the egg of an oviparous squamate species, and B: the uterus of a viviparous squamate species. The allantoic cavity is surrounded by the allantois and the yolk is enclosed within the vitelline membrane. Apposition of the chorion and the allantois forms the chorioallantoic membrane. Simple placentation forms within the viviparous species when the chorioallantoic membrane (forms chorioallantoic placenta) and vitelline membranes (forms yolk sac placenta) lie adjacent to the uterine epithelium. (Modified from diagram in Guillette, 1993)

Regional hypertrophy of uterine epithelial tissues may occur, as in the development of the omphaloplacenta (yolk-sac placenta; Stewart, 1992) in the snake *Virginia striatula*. The uterine epithelial cells of the omphaloplacenta (in the region of the isolated yolk mass) were initially cuboidal with a rich vascular system. As the allantois extended into the isolated yolk cleft, the omphalallantoic...
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The placenta was formed. The uterine epithelial cells in this region then hypertrophied to form columnar cells arranged on pilae (mucosal folds) which extended from the lamina propria (Stewart, 1990; Stewart, 1992). Capillaries lay at the base of the mucosal columns. The uterus apposed to the other placental regions in this species was heavily vascularised with a squamous epithelium (Stewart, 1990; Stewart, 1992).

Only a few squamate species studied to date exhibit advanced forms of placental interaction (Blackburn et al., 1984; M. Thompson and Stewart, 1994). These species are predominantly matrotrophic and ovulate small eggs with little or no yolk. In Chalcides chalcides (Blackburn, 1993b) and Mabuya bistriata (Vitt and Blackburn, 1991), a specialised region of the chorioallantoic placenta called the placentome was present. The uterine mucosa underwent hypertrophy to form branching folds or outgrowths which protruded into deep invaginations of the chorioallantois. The interdigitation of uterine and foetal tissues at the placentome increased the surface area available for physiological exchange (Blackburn, 1993b). It was considered most likely that nutrient provision by the placentome is histotrophic, meaning that the hypertrophied uterine epithelium secretes material synthesised from precursors in the maternal blood system (Blackburn, 1993b). In the remaining chorioallantoic placenta of M. bistriata which is not part of the placentome, chorionic crypts were found (Vitt and Blackburn, 1991). These were invaginated pits lined by microvillous columnar cells which were opposed to openings of uterine glands. Secretory material was evident within the crypts and the cytoplasm of the crypt epithelium contained inclusions which stained identically to uterine secretions.

As yet, the particular functions of uterine regions associated with different parts of the placenta are unknown. Also unknown is the mechanism which causes the differentiation of the uterus to form placental associations. It is not unreasonable to expect that local paracrine action by the developing extra-embryonic membranes act to cause differentiation. This is a potential area for the action of growth factors.

Obviously vital to placental exchange is the uterine blood supply. Vascularity peaks during gestation (Guillette and Jones, 1985b) and gravidity (Masson and Guillette, 1987) in viviparous and ovi-oviparous species. Masson and Guillette (1987) hypothesised that major changes in uterine vascularity may not be required for the evolution of simple placentation in reptiles. Guillette and Jones
(1985b) suggested that increased oviductal vascularity seen in viviparous species may be stimulated by hypoxia of the uterus caused by the embryo. As the embryos remove oxygen from the uterine tissue, other existing blood vessels would be stimulated to open and new ones could form.

A placenta is vital for water and gas exchange, and potentially inorganic and organic nutrient exchange, between maternal and foetal tissues (Yaron, 1985). Unfortunately, little is known of the exact locations of the physiological exchange or how the oviduct mediates the transfer. Information available to date concerning placental transport in viviparous species is based on comparisons of the composition of ovulated eggs with neonates (J. Thompson, 1981, 1982; Stewart et al., 1990; Stewart and Thompson, 1993) and on the uptake by embryonic tissues of labelled ions or amino acids and their metabolites (Veith, 1974; J. Thompson, 1977; Swain and Jones, 1997). Blackburn (1994) discussed the relative merits of different methodologies. He concluded that comparisons of the composition of eggs and neonates provides the most reliable method of quantifying the source of nutrients for development. The studies using both methods suggest that both organic and inorganic ions can cross the placenta. These experiments have all been undertaken in relatively lecithotrophic species, suggesting transfer of at least some inorganic or organic compounds is a common feature of viviparous squamates (Blackburn, 1994). In matrotrophic species, however, considerably more physiological exchange must occur. For example, in Mabuya heathi, a skink which ovulates the smallest known reptilian egg (≈ 1 mm diameter), placental transport accounted for >99% of dry mass increase in neonates (Blackburn et al., 1984).

In mammals, the placenta is a well-known endocrine organ, but as yet, there has been little consideration of the potential of the reptilian placenta to synthesise hormones. As with the mammalian placenta (Strauss et al., 1996), the reptilian placenta is ideally situated to utilise steroid precursors contributed by either the mother or the foetus, as well as to be influenced by hormones from either the mother or the foetus. The yolk supply available to the embryo, which was found to contain androstenedione, estradiol and testosterone in Alligator mississippiensis (Conley et al., 1997), may also supply hormones capable of crossing the placental barrier. In Sceloporus jarrovi, several events occurred in the fourth month of pregnancy: the corpus luteum began to degenerate, rapid embryonic growth began, the chorioallantoic placenta formed, and there was a marked increase in plasma progesterone concentrations (Guillette et al., 1981).
This suggested that the corpus luteum was not the only source of progesterone during pregnancy in *S. jarrovi*. Progesterone measured at this time may be secreted by the corpora atretica or the chorioallantoic placenta. The uterus is already known to be a potentially steroidogenic tissue (see previous discussion), so the potential production of hormones (including growth factors) by placental tissues needs addressing.

In summary, most viviparous squamates have a simple placental arrangement which is essential for water and gas exchange, nutrients being supplied predominantly by the yolk. More advanced forms of placentation are found, however, with regional differentiation of the uterine tissues associated with the developing extraembryonic membranes. Little is known concerning the potential nutrient transfer across the placenta, nor the potential for endocrine interactions between maternal and foetal tissues.

**Oviposition/Parturition**

At the completion of gravidity or gestation, the oviduct plays a role in oviposition or parturition. These processes are achieved by rhythmic contractions of the oviductal muscularis and they involve various hormones, including arginine vasotocin (AVT) and prostaglandins (PGs), as well as neural factors (Guillette *et al.*, 1991b). Females exhibit seasonal variations in spontaneous contractions of the oviduct with maximal contractility occurring during gravidity (Abrams Motz and Callard, 1988). The trigger for the contractions that cause oviposition or parturition is a topic which has received considerable attention in the reptilian literature with several detailed reviews available (Yaron, 1972; La Pointe, 1977; R. Jones and Guillette, 1982; Guillette *et al.*, 1991b; R. Jones and Baxter, 1991).

The neurohypophysial hormone, AVT, appears to play a major role in the processes of oviposition or parturition. In oviparous and viviparous species, AVT has been shown to induce oviductal contractions *in vitro* (La Pointe, 1969; La Pointe, 1977; Guillette and Jones, 1980; Cree and Guillette, 1991; Fergusson and Bradshaw, 1992; Guillette *et al.*, 1992) and also to induce oviposition (Guillette and Jones, 1982) or parturition (Guillette, 1979; Guillette and Jones, 1982; Cree and Guillette, 1991). Maximal sensitivity of the oviducts to AVT occurred at approximately the same temperature as the preferred body temperature for each species examined by La Pointe (1977).
In the sea turtles _Lepidochelys olivacea_ and _Caretta caretta_, AVT and neurophysin (prohormone) levels were low in animals right up until oviposition (Figler et al., 1989). These levels were consistent with the hypothesis that the AVT-neurophysin complex is released from the neurohypophysis during nesting and that AVT is the physiological regulator of oviductal contractions in sea turtles. Similarly, in the tuatara _Sphenodon punctatus_, high levels of AVT were recorded during oviposition (Guillette et al., 1991d). However, in the viviparous lizard, _Tiliqua rugosa_, plasma AVT rose approximately 30 days prior to parturition (Fergusson and Bradshaw, 1991). If, as suggested, AVT is the physiological regulator of oviductal contraction, another factor(s) must prevent parturition until the appropriate time. Adrenergic and peptidergic innervation of the oviduct was analysed in _Sceloporus jarrovi_ (Rooney et al., 1997) and showed seasonal changes. Innervation will undoubtedly contribute to the mechanisms controlling oviposition/parturition. The rise in AVT in _T. rugosa_ was concurrent with a decrease in plasma progesterone from high levels during mid-pregnancy to basal levels at parturition as the corpus luteum degenerated (Fergusson and Bradshaw, 1991).

Oviposition is often correlated with regression or surgical removal of the corpus luteum in oviparous species (Klicka and Mahmoud, 1977; H. Cuellar, 1979), which led to the suggestion that luteal regression induces AVT secretion (Guillette and Jones, 1982; Jones and Guillette, 1982). The role of the corpus luteum in parturition in viviparous species is not so clear. Considerable difference in the timing of luteal regression exists between species, as does the response to deluteinisation (Xavier, 1987; I. Callard et al., 1992).

AVT's actions may also be modified by sex steroids. In _T. rugosa_ (Fergusson and Bradshaw, 1992), the strength of uterine contractions _in vitro_ was reduced by pretreatment _in vivo_ with progesterone or estradiol, although the frequency of AVT-induced contractions was enhanced by estradiol pretreatment. Although the strength of AVT-induced contractions was not significantly different between pregnant and non-pregnant females, spontaneous rhythmic contractions were present only in pregnant females. Ovariectomy did not affect the spontaneous or the AVT-induced contractions in _T. rugosa_ (Fergusson and Bradshaw, 1992).

Precursor compounds (eicosatrienoic acid and arachidonic acid) for prostaglandins (PGs) were identified in the oviduct of the viviparous lizard _Sceloporus jarrovi_, and the oviduct _in vitro_ produced compounds which
migrated alongside matching PG standards using thin layer chromatography (Guillette et al., 1988). Responsiveness of oviducts to arachidonic acid in vitro indicated that the oviducts were capable of producing PGs (Guillette et al., 1991c). Uteri of Alligator mississippiensis secreted PGF and PGE in vitro (Dubois and Guillette, 1992). Secretion of PGs was lowest during gravidity, but increased dramatically during oviposition. PG levels were also elevated around the time of oviposition in the tuatara (Guillette et al., 1990). PGs can be produced by the corpus luteum as well as by oviductal tissue (Gobbetti et al., 1993). Amounts and types of PG produced by the corpus luteum of Podarcis sicula sicula varied depending on the reproductive stage of the female.

Guillette et al. (1991c) treated gravid females of several lizard species with PGF2α, PGE2 or arachidonic acid (PG precursor) in vivo. No dosage induced oviposition in any of the species examined. However, if oviducts were isolated in culture with PGF2α or arachidonic acid, oviducts did oviposit. Guillette et al. (1991b) hypothesised that PGs can stimulate oviposition in oviparous species, but that the action of PGs may be inhibited by oviductal innervation.

In late pregnant females of the viviparous lizard Sceloporus jarrovi, administration of PGF2α, PGE2 or arachidonic acid in vivo, or to cultured oviduct in vitro, stimulated parturition within 2 h (Guillette et al., 1992). Administration of the above compounds into mid-pregnant females elicited no response in vivo. In oviducts cultured in vitro from mid-pregnant females, contractions were stimulated by PGF2α, but not by arachidonic acid. Similarly, AVT stimulated parturition in vitro in late pregnant but not mid-pregnant females. In S. jarrovi, where progesterone falls markedly prior to parturition, subcutaneous implants of progesterone significantly delayed parturition (Guillette et al., 1991a). Implants of indomethacin, which inhibits synthesis and release of prostaglandins, also delayed parturition as well as disrupting the normal birth process.

In a population of geckos from the Hoplodactylus maculatus species complex, AVT, but not PGF2α induced parturition in vivo in late pregnant females (Cree and Guillette, 1991). Pretreatment of females with β-adrenoreceptor antagonist before administration of PGF2α stimulated parturition. Uteri treated in vitro contracted in response to both AVT and PGF2α. Pre-exposure of uteri to a β-adrenoreceptor agonist caused relaxation of tissue. However, pre-exposure did not prevent contractions induced by AVT, but did prevent contractions induced by PGF2α. The results support the hypothesis that β-adrenergic stimulation
inhibits the contractile response to PGF$_{2\alpha}$ by H. maculatus (Cree and Guillette, 1991). β-adrenergic inhibition of oviposition was also noted in the lizard Anolis carolinensis (Jones et al., 1983b; Summers et al., 1985). The uterus was found to respond to hormonal stimulation once a β-adrenergic agonist was administered or the uterus was surgically denervated.

The lizard Anolis carolinensis has provided a more complicated puzzle (Guillette and Jones, 1985a). A. carolinensis ovulates an egg from alternative ovaries approximately every 14 days followed by unilateral uterine contraction and oviposition. AVT did not stimulate oviposition (Guillette and Jones, 1982), although oviductal contractions were stimulated by AVT in vitro in ovarietomised females (Guillette and Jones, 1980). In naturally cycling females, oviductal contractions stimulated by AVT occurred in oviducts ipsilateral to an ovary in which the corpus luteum was degenerating or absent (Jones et al., 1982; Jones and Guillette, 1982). If a corpus luteum was present on the ovary on the same side as the oviduct tested, AVT failed to stimulate contraction. Unilateral deluteinisation removed the unilateral inhibition of oviductal contraction. The authors suggested that inhibitory substances may have been secreted by the corpus luteum and delivered to the ipsilateral uterus via small utero-ovarian blood vessels (Jones et al., 1982; Jones et al., 1983a).

In mammals, relaxin (an insulin-like peptide) reaches peak plasma concentrations just prior to birth and causes cervical softening and relaxation of the pelvic ligaments allowing the pelvis to stretch and expand during parturition (Sherwood, 1988). Unfortunately, almost nothing is known concerning the actions of relaxin in reptilian species. In the turtle Chrysemys, relaxin significantly decreased the interval between contractions of the uterine muscularis (Sorbera et al., 1988). Similarly, in sharks, relaxin depresses contractile activity of the uterine muscularis (Sorbera and Callard, 1987). In the viviparous dogfish Squalus acanthias, relaxin increased the cervical cross-sectional area resulting in premature birth (Koob et al., 1984). The response to relaxin was specific to the cervix, not being observed in the anterior uterine constriction. Guillette et al. (1991b) suggested that the posterior oviduct of vertebrates (cervix, uterovaginal junction) may act to retain the eggs or embryos in utero because it is under separate control from other regions of the oviduct. The possibility of a functionally separate cervix (vagina) and the potential actions of relaxin needs further research within the reptiles, as well as in other vertebrate groups.
In *Lacerta vivipara*, plasma corticosterone levels reached a peak in late gestation (Dauphin-Villemant *et al.*, 1990). In addition, daily injections of corticosterone during late gestation significantly delayed the timing of parturition in *Lacerta vivipara* (Dauphin-Villemant *et al.*, 1990) indicating that corticosterone may be involved in the parturition process. However, in females from a population of the *Hoplodactylus maculatus* species complex (*H.* n. sp. "Otago"), corticosterone plasma concentrations did not vary between females in four reproductive conditions (vitellogenic, mid-pregnant, late pregnant and spent). These results do not support a role for corticosterone in maintaining gestation in this species (Girling and Cree, 1995). To my knowledge, no further information concerning the possible role of corticosterone in parturition is available.

It seems, therefore, that the major endocrine and neurological players in the oviposition/parturition process may have been identified, but the details of all the direct or indirect links between mechanisms causing oviductal contraction are still not understood. Guillette *et al.* (1990) summarised the probable actions of the main players as follows: AVT and prostaglandins induce oviductal contractions needed for oviposition/parturition, β-adrenergic neurons inhibit oviposition/parturition, and AVT seems to be able to override the β-adrenergic inhibition. The possible actions of relaxin and corticosterone need to be determined.

**FUTURE RESEARCH**

Within the field of study addressing the structure and functions of oviductal tissues, there is considerable scope for further research. Although it is not possible to identify every conceivable question that needs addressing, certain key areas stand out. These key areas, some of which have been alluded to in the above discussion, are collated and restated below.

The potential functions of the infundibulum have not been analysed in any detail. The infundibulum is the most anterior region of the oviduct and it exhibits various epithelial cell characteristics, such as apical protrusions and blebbing, which are not understood. The infundibulum is known to be secretory; oviductal secretions coat the egg as soon as it enters the infundibulum and epithelial cells contain various secretory granules. What is the nature of these secretions? As a working hypothesis, I suggest that the infundibulum secretes mucus necessary for lubrication as the egg enters the oviduct. This hypothesis
does not preclude other possibilities which need clarification, namely that the infundibulum may play a role in albumen secretion and that it may be a region which secretes growth factors. The infundibulum is ideally placed to secrete materials directly onto the ovulated egg prior to albumen and shell secretion.

The traditional view of estradiol-mediated oviductal development has been challenged, particularly in certain species. It appears androgens may play a larger role in oviductal development than has been previously realised. Further characterisation of androgen receptor systems and aromatase activity in various species will be invaluable. I hypothesise that, in certain species (such as the garter snake, *Thamnophis sirtalis*, studied by J. Whittier and her colleagues [Whittier et al., 1987, 1991; Whittier, 1992]), after an initial burst of estradiol at the beginning of vitellogenesis which triggers oviductal development, androgens are capable of maintaining the hypertrophied oviduct. Whether the androgens are acting directly on the oviduct or are being aromatised to estradiol would need investigation.

Analysis of the actions of growth factors will undoubtably add to our understanding of the reptilian oviduct and the use of models based on information available for mammals will provide useful working hypotheses. For instance, in mammals it is believed that estradiol-induced uterine development is mediated by EGF. This was based on the observation that hypertrophy of the uterine epithelium observed in vivo could only be elicited in vitro if epithelial tissue was cultured in combination with stromal tissue. This suggested that paracrine factors were in action. Use of in vitro models in reptilian species will be important in determining the process by which estradiol and growth factors stimulate oviductal development. For example, based on the above mammalian data, do reptilian epithelial cells cultured in vitro hypertrophy in response to EGF, but not to estradiol?

Another example where a model based on mammalian data can be applied to reptiles involves IL-1. In mammals, IL-1 induces production of prostaglandin-E2. In the lizard *Chalcides chalcides*, immunoreactivity for IL-1 was strongest just prior to parturition. I hypothesise that production of IL-1 at this time triggers the production of prostaglandins necessary for egg-laying/parturition.

Analysis of egg shelling in reptiles is hampered by our lack of knowledge concerning calcium secretion. The current hypothesis that the epithelium is the
most likely source of shell calcium makes intuitive sense. The epithelium, which lines the lumen of the oviduct, is ideally placed to secrete an even layer of calcium over the entire egg. However, further specialised techniques will be required to confirm this hypothesis. As the mechanism of calcium secretion in oviparous species is unknown, it is difficult to hypothesise how the process has been lost/changed in viviparous species. In the continuing debate concerning the evolution of viviparity, any information concerning the control of calcium secretion will be vital.

Research analysing the potential nutrient transfer by reptilian placental tissues has begun but, as yet, little consideration has been given to the potential hormonal actions of the squamate placenta. In viviparous species, the lifespan of the corpus luteum (the major source of progesterone) varies and in some species it degenerates well before parturition. The hypothesis by Guillette et al. (1981) that the placenta is another source of progesterone should be investigated. In certain viviparous squamate species, development of the placenta is associated with differential development of the uterus. I hypothesise that growth factors or sex steroids produced by the extraembryonic membranes are responsible for the differential development. Use of immunocytochemical techniques will be useful to identify the presence and distribution of various hormones in placental tissues. These techniques will be most useful when used along with in vitro techniques to characterise the type and quantity of hormones produced by placental tissues.

The hypothesis by Guillette et al. (1991) that the vagina may act as a functional cervix with separate control from other oviductal regions needs further consideration. Again, in vitro techniques will be useful to identify differential response to AVT, prostaglandin, relaxin and neural factors among the different oviductal regions.
CONCLUSIONS

The reptilian oviduct is a diverse tissue with various functions depending on the reproductive mode of the species in question. The infundibulum initially receives the ovulated egg and oviductal secretions immediately cover the egg. The exact nature of these secretions is unknown. The uterine tube is responsible for secretion of the albumen layer found in crocodilians and turtles. The role of the uterine tube in lepidosaurians is inconclusive as no distinct albumen layer is present in their eggs. The uterus secretes the eggshell (both shell membranes and the calcareous components) in oviparous species. Mechanisms of shell secretion, particularly in regard to the production of the calcareous layer, are not well understood. In viviparous species, associations of the uterus with the extraembryonic membranes form the placenta. We still know little about what crosses the placental barrier, particularly potential hormone and growth factor transport. At the completion of gravidity/gestation, the oviduct musculature contracts to cause oviposition/parturition, a procedure which requires AVT, PGs as well as neural factors.

There is considerable potential for future research into this fascinating organ. In particular, improved techniques to identify endocrine, molecular and biochemical mechanisms acting in the oviduct will provide useful insight.
Chapter Eight

Conclusions

General Conclusions
GENERAL CONCLUSIONS

The research in this thesis has provided detailed structural and ultrastructural information about the oviduct from wild, and experimentally manipulated, female geckos. This extends the limited information previously available for gekkonid species. Many of my hypotheses about oviductal structure and function were supported, although some unexpected results were obtained in relation to the effect of ovariectomy and hormone treatment. My work has highlighted the need for additional research from several perspectives, including determining the function of several ultrastructural features of the oviduct described in this thesis.

The general structure of the oviduct from the gekkonid species analysed in this thesis was typical of that in other squamate reptiles (For instance: Palmer et al., 1993; Perkins and Palmer, 1996). The oviduct was made up of five regions: (starting anteriorly:) infundibulum, uterine tube, isthmus, uterus and vagina. In the viviparous New Zealand gecko Hoplodactylus maculatus, differences in oviductal structure among four different reproductive stages were described. Changes were observed in epithelial cell height (in the four regions analysed: infundibulum, uterine tube, uterus and vagina) with maximum height occurring during vitellogenesis and a drop in height following ovulation. There were also changes in the ratio of ciliated to non-ciliated cells, and in the surface area of epithelium occupied by ciliated cells, among the four reproductive stages. My results indicate that the size and number of ciliated and non-ciliated cells may change in a predictable manner over a reproductive cycle.

The uterus exhibited the most obvious changes over the four reproductive periods analysed in H. maculatus. During vitellogenesis, the uterus was a thick-walled region with few mucosal (shell) glands. Following ovulation, the uterus was highly stretched due to the presence of a large yolky egg. By late pregnancy, uterine tissues formed placental interactions with extraembryonic membranes.

The uterus also exhibited the most obvious differences in structure among four geckos exhibiting differing parity modes and eggshell types. In viviparous H. maculatus and Hoplodactylus divaucelii, few mucosal glands (responsible for secretion of the shell membrane) were present in the uterus. This is consistent with other viviparous squamates analysed (for instance: Guillette and Jones, 1985b) and it is also consistent with the fact that the shell membrane seen
surrounding the egg of *H. maculatus* during the early stages of pregnancy was very fine. The membrane was no longer visible in late pregnant females, but the mechanism by which the shell membrane was lost is unknown. It is unknown whether *H. duvaucelii* has a shell membrane during pregnancy. The uterus of the oviparous species *Saltuarius wyberba* (which is thought to produce a soft, parchment-like eggshell) and *Hemidactylus turcicus* (which produces a hard, calcareous eggshell) had numerous uterine mucosal glands and this is consistent with results described for other oviparous species (for instance: Guillette *et al.*, 1989). The difference in the number of uterine mucosal glands is a characteristic difference between oviparous and viviparous species and determining the mechanism of gland loss in viviparous species will provide important clues concerning the evolution of viviparity. The staining intensity of the glands, and the number of secretory granules they contained, differed between the two oviparous species. The differences may be due to the variation in eggshell types produced by these animals. Future research to elucidate the reasons for the differences between glands needs to analyse the chemical nature and quantity of material produced by the glands of different species. Differences were also seen in the epithelial layer of the two oviparous species, but whether this relates to differences in the amount of calcareous material needed for the different eggshell types is unknown. As yet, the source and mechanism of calcium secretion required for eggshell production in reptiles has not been determined with certainty.

Some differences in oviductal structure observed between the different geckos did not directly relate to differences in parity mode or eggshell structure. I hypothesised that this may be due to the different time periods until ovulation of the females in question. Although all females analysed were in late vitellogenesis, it was not known if the females collected from any one species were one day, or one week (for instance) away from ovulation. The period just prior to ovulation is potentially critical with last-minute development and changes in preparation for gravidity/gestation. Future studies analysing the changing structure of the oviduct over a reproductive cycle should consider analysing the period just prior to and during the process of ovulation.

The seasonal development observed in the oviductal structure of reptiles in preparation for gravidity/gestation is believed to be stimulated by secretion of estradiol from the ovary during vitellogenesis. Oviductal hypertrophy can be induced in non-reproductive and ovariectomised females following treatment
Conclusions

8.4 with exogenous estradiol (For instance: Veith, 1974; Mead et al., 1981). In a study with the oviparous gecko H. turcicus, I aimed to analyse the effects of exogenous estradiol on uterine tissues in ovariectomised females over a two week treatment period. The results confirmed that exogenous estradiol causes uterine hypertrophy in ovariectomised females and provided additional ultrastructural information (TEM) over the period of treatment. Ovariectomised females exhibited uterine regression with a low cuboidal epithelium, little differentiation and few, if any, secretory granules. Following estradiol treatment, there was a significant increase in epithelial cell height, the epithelium was fully differentiated and non-ciliated cells contained numerous secretory granules. Mucosal glands exhibited two cell types which had not been distinguished in naturally vitellogenic females. Whether these cell types represent a natural stage in gland development or an artefact of experimental manipulation is not known.

An experiment administering exogenous hormones to ovariectomised females was also carried out with H. maculatus. The aim of the study was to analyse the effects of exogenous progesterone on oviductal structure in ovariectomised, estrogen-primed females. However, the results obtained from treatment with estradiol, or estradiol and then progesterone, were confounded because of the unexpected oviductal structure exhibited in ovariectomised females treated with vehicle solution only. No oviductal regression was noted in the vehicle-only females and their oviducts resembled those seen in naturally vitellogenic females. Future research needs to determine the reasons for these unexpected results. Possible working hypotheses were discussed and included a suggestion that androgens produced by the adrenal gland may be aromatised to estradiol by the brain or by the oviduct itself. Additionally, androgens may act directly on the oviduct thereby maintaining oviductal hypertrophy.

Oviductal tissue analysed in this thesis, from wild females and females surgically and hormonally manipulated, has been compared using both light and electron microscopy. Light microscopy provided a general overview of oviductal tissues and gave an idea of the chemical nature of secretory material. For instance, epithelial cells from differing oviductal regions exhibited different carbohydrate staining properties. Electron microscopy (particularly transmission) allowed me to look at ultrastructure and sub-cellular characteristics not visible using light microscopy. Previous to this thesis, little ultrastructural information was available for the reptilian oviduct. I noted features such as bleb cells and other apical protrusions during my research, particularly in the infundibulum. The
function of the various protrusions are not known; however, similar protrusions, which are predominantly progesterone-dependent, have been identified in mammalian species. The use of in vitro culture techniques will be a necessary tool to determine the function and hormonal control of bleb cells and other apical protrusions; indeed, I believe in vitro cell culture techniques will be invaluable to determine the actions of hormones (including growth factors) on particular oviductal tissue types (such as epithelium) and particular oviductal regions.

The stimulatory effects of estradiol on oviductal tissue are believed to be mediated by growth factors. I attempted to use immunocytochemistry (ABC method) to determine the presence and distribution of IGF-I in the oviduct of gekkonid species. Unfortunately, validations were not satisfactorily completed. However, once technical difficulties are overcome, this technique will be valuable for future researchers to determine the distribution of various growth factors and other antigens of interest. Immunocytochemistry will most appropriately be used with molecular techniques to characterise the antigen of interest.

The review concluding the thesis outlines the diverse functions of the reptilian oviduct and places results from my studies in context with the current literature. Although considerable work has already been done, I identified several areas for which future research will provide valuable information: the functions of the infundibulum have not been considered in detail, the potential actions of androgens on oviductal tissue need addressing more fully, the literature concerning the actions of growth factors in mammals will provide valuable working hypotheses for research into reptilian species, the mechanism by which calcium for the eggshell is secreted by the reptilian oviduct is unknown and the potential hormonal interactions facilitated by placental tissues have not yet been investigated.

The reptilian oviduct has proved a fascinating subject for study. The oviduct's diverse functions provide for considerable variation in study topics. Reptiles themselves exhibit considerable variation in aspects of reproduction, be it parity mode, eggshell type, or the source of nutrients supplied to developing embryos. The diversity of study topics related to the reptilian oviduct and the continuing development of new scientific tools means that the oviduct will long be a topic of research interest.


References


References


References


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