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Reproductive Biology of Indian Reptiles

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Various aspects of reproductive biology such as sex differentiation, cyclic changes in spermatogenesis, ovaries (follicular development, follicular kinetics, etc), sex steroids and accessory reproductive structures, clutch patterns, role of fat bodies in reproduction, endocrine and environmental control of reproduction have been reviewed with a focus on the reproductive strategies. In addition, the gaps in our understanding of the diverse reproductive strategies, aspects of captive breeding and management are discussed to shed light on the state of art.

Key Words: Reproductive Biology, Indian Reptiles, Sex differentiation, Testicular cycle, Sex steroids, Ovarian cycles, Clutch patterns, Regulation of gonadal cycle, Abdominal fat bodies and Reproduction

Introduction

Reptiles are the first vertebrates that successfully adapted to life on land. Evolution of internal fertilization and cleidoic eggs is a major adaptation to terrestrial life that involves water conservation through protective membranes and adjustment of nitrogen metabolism (Packard 1966, Romer 1971). Although chelonians, crocodiles and a few ophidians are secondarily adapted to life in water, for reproduction they come on land to lay eggs. Information on the gonadal sex differentiation, gonadal cycles and their control mechanisms is largely available for reptiles from outside India (Fox 1977, Duvall et al. 1982, Licht 1984, Whittier & Crews 1987, Vitt 1992, Crews 1996, Pieau 1996, Jeyasuria & Place 1998, Pieau et al. 2001). Like in most other vertebrates, reptiles also concentrate reproduction at a season that is most favourable for growth and survival of their offspring. Environmental variables such as temperature, photoperiod, rainfall, food abundance, and socio-sexual factors play an important role in coordinating reproductive events. The reptiles of temperate region are strictly seasonal breeders, while those from tropics exhibit a continuum from acyclic to cyclic breeding (Angilini & Ghiara 1984, Saidapur 1989). The physiological/ endocrine mechanisms that mediate the influence of proximate environmental factors in reproduction in reptiles are not well understood.

Though around 456 species of reptiles inhabit the Indian subcontinent (J.R.B. Alfred, Director, ZSI, Kolkota, personal communication), many aspects of their reproduction are poorly studied. Information on reproductive biology in Indian reptiles is mainly available for some lacertilians. The present article therefore reviews the state of art of different aspects of reproductive biology of Indian reptiles with a major focus on the lizards.

Sex Differentiation

Gonadal Sex Differentiation

Studies on gonadal sex differentiation are carried out in a number of reptilian species belonging to different orders (reviewed by Crews 1996, Pieau 1996, Jeyasuria & Place 1998, Pieau et al. 2001). Among the Indian reptiles, studies on the gonadal sex differentiation are limited to the garden lizard *Calotes versicolor* (Gaitonde & Gouder 1984, Doddamani 1994). At oviposition, the eggs of *C. versicolor* normally have embryos at stage 27-28 and at hatching the embryo is at stage 42 (Muthukaruppan et al. 1970). In stage 27 embryo, distinct genital ridges are seen on either side of the dorsal mesentery adjacent to the mesonephric

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kidneys. The indifferent gonad at embryonic stage 31-32 is distinguished into cortex and medulla. According to Gaitonde and Gouder (1984), gonadal sex differentiation occurs in one-day-old hatchling but distinct ovaries and testes are formed 15-days later. On the other hand, Doddamani (1994) reported that the sex differentiation of gonads occurs at embryonic stage 34, and oogenesis begins by stage 35 in the same species. The ovaries are distinguished at this stage by the formation of distinct cortex with 3-4 layers of oogonia while testes become distinguishable in stage 36 embryos with distinct seminiferous cords. Gaitonde and Gouder (1984) incubated eggs at ambient temperature (~29°C) while Doddamani (1994) incubated eggs at a constant temperature (29° – 30°C). This might be the reason for the discrepancy of results in the two studies. It is pertinent to note that incubation of *C. versicolor* eggs at higher temperature advances gonadal sex differentiation (Ganesh & Raman 1995) and hatching date (Ganesh & Raman 1995, Radder et al. 2002a). Unfortunately, studies of Gaitonde and Gouder (1984), and Doddamani (1994) do not mention the duration of incubation till hatching.

Factors Regulating Gonadal Sex Differentiation

In several reptilian species, sex determination is known to be dependent on the temperature to which the eggs are exposed. Temperature dependent sex determination (TSD) has been documented in 28 species of turtles, some lizards and all crocodiles (Crews 1996, Pieau 1996, Jeyasuria & Place 1998, Pieau et al. 2001). The temperature profiles of sex differentiation in these orders, however, vary. The mechanisms underlying TSD in reptiles are still not fully understood (Pieau 1996, Bowden et al. 2000, Pieau et al. 2001). Among the reptiles from India, the role of temperature in sex determination is studied in *C. versicolor,* olive ridley turtle, *Lepidochelys olivacea* and the salt-water crocodile, *Crocodilus porosus*. Generally the phenomenon of TSD is observed among species that lack sex chromosomes. However, sex determination is not temperature dependent in *C. versicolor* (Ganesh & Raman 1995) though sex chromosomes are not distinguishable in this species (Singh 1974). The application of testosterone (T) to embryos at stages 28, 30 and 33 resulted in the production of a greater number of males while estradiol-17β (E_2) did not induce feminization (Ganesh & Raman 1995). Ganesh et al. (1999) showed that between 5-15 day of embryonic development the gonads at ambient temperature are sensitive to T for sex reversal. Exogenous application of T to embryos at ambient temperature (28°C) resulted in male hatchlings. In contrast, at higher incubation temperature (33°C), T application to the eggs produced individuals of both the sexes. Interestingly, the eggs treated with dehydrotestosterone (DHT) at 33°C produced males. Likewise, eggs at 33°C treated with T and applied with aromatase inhibitor (CGS 16949A) on day 15 or 25 of incubation resulted in production of males. The authors therefore suggest that high temperature *per se* has no direct effect on sex determination in *C. versicolor*. They further concluded that high temperature might have a stimulatory effect on aromatase activity, leading to the conversion of exogenously applied T into estrogen thus permitting ovarian differentiation in genic females. Probing genome of this lizard with the human Y-linked SRY and ZFY genes showed a sex specific bias in their distribution. The SRY probe hybridized to all males but to less than half among the females. On the other hand, ZFY hybridized to both the sexes (Ganesh et al. 1997). It is apparent that human SRY gene in *C. versicolor* exhibits a male distribution bias. Recently, Choudhary et al. (2000) have cloned and sequenced an orthologue of human *SOX9* gene in *C. versicolor*. This gene exhibits a cell specific expression in the genital ridge of embryonic testis (medulla) suggesting a vital role for *CaSox9* gene in determination and/or differentiation of testis.

In the turtle, *L. olivacea,* sex chromosomes are not discernible and TSD is reported to operate. The females are produced ≥ 30°C while males are produced at ≤ 28°C. Interestingly, at 29.5°C both males and females are produced (Mohanty-Hejmadi & Diamond 1986). In the crocodile, *C. porosus* also, TSD is reported to operate (Mohanty-Hejmadi et al. 1999). The eggs incubated at 33°C and 34°C hatched as females and males respectively. There is no information about the sex chromosomes in this species.

The Male Reproduction

Morphology of the Testis

All reptiles possess a pair of compact testes in the abdominal region. The testes of the fussorial blind snakes, *Typhlops* and *Leptotyphlops* are multilobed. The testis is covered by connective tissue capsule, tunica albuginea, and attached dorsally to the body wall by a mesorchium. Reptilian testis consists of convoluted seminiferous tubules and interstitial tissue. Seminiferous tubules contain permanent germinal epithelium and Sertoli cells.

Spermatogenesis

The spermatogenesis is noncystic type but cytoplasmic bridges interconnect the germ cells of a given generation (derived from a spermatogonium) to form heterokaryon. It is not known whether cellular associations and seminiferous epithelial cycles as seen in mammals occur in reptiles (Roosen-Runge 1977, van Tienhoven 1983, Saidapur & Shanbhag 1999). The duration of spermatogenesis from spermatogonia to sperm is not known for any Indian reptiles. However, in soft-shelled turtle, *Lissemys punctata punctata*, studies involving ^{3}H-thymidine administration followed by autoradiography have shown that formation of young spermatids from leptotene spermatocytes takes about 12 days (De & Maiti 1987a). Based on the changes in the nuclear shape (using PAS-hematoxylin), 16 stages of spermiogenesis are recognized in the turtle.

Sertoli Cells

Distinct Sertoli cells border inner side of the basement membrane of the seminiferous tubules in association with germ cells in various stages of spermatogenesis. The Sertoli cells are easily identified under light microscope in the regressed testis by their characteristic triangular shaped nuclei (figure1A). Sertoli cells of *C. versicolor* (Gouder & Nadkarni 1979), *Psammophilus dorsalis* (Sarkar & Shivanandappa 1989) and in *L. p. punctata* (De & Maiti 1987b) exhibit 3β- HSDH enzyme activity. Scanning electron microscopic (SEM) study on Sertoli cells of *L. p. punctata* revealed that these cells are broad at the basal end with cytoplasmic processes tapering towards luminar end. The Sertoli

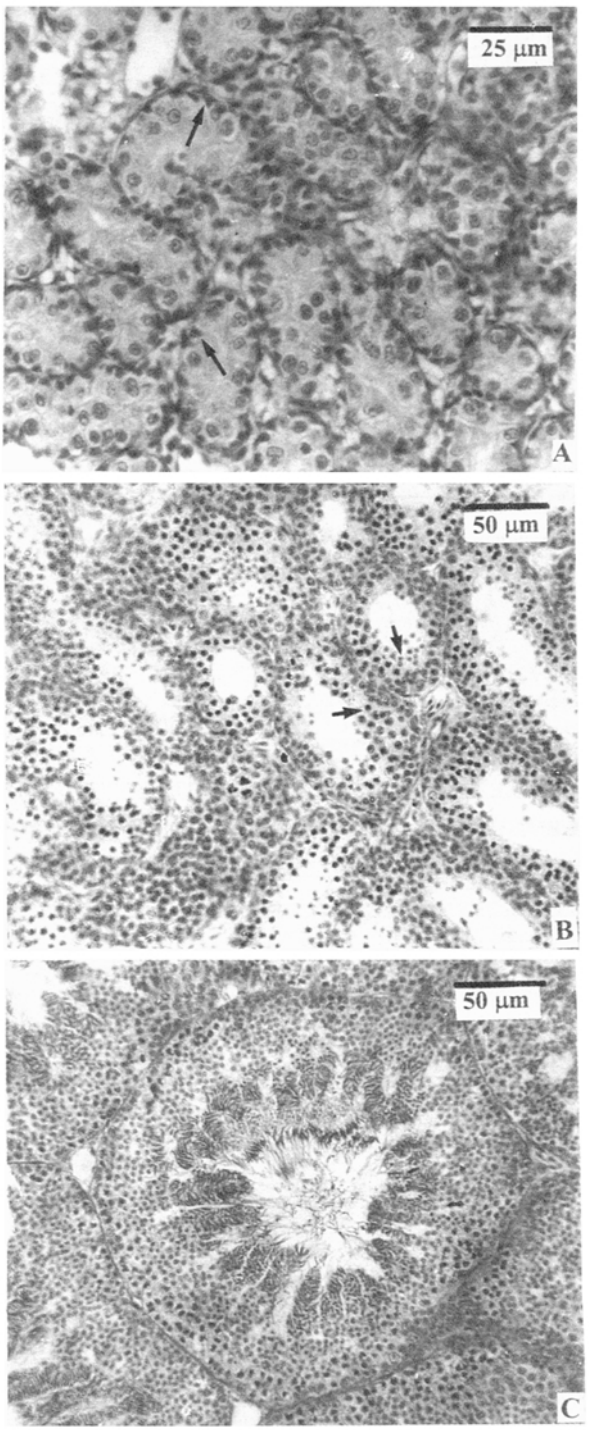

Figure 1 A-C: T S of testis of *C. versicolor* **A**, Testis during postbreeding phase: Note small sized seminiferous tubules lined with a few spermatogonia and Sertoli cells (arrows); **B**, Testis during recrudescence phase showing more number of germ cells upto primary spermatocytes (arrows). Note the increase in size of seminiferous tubules compared to that in A; **C**, Testis during breeding phase showing enlarged seminiferous tubules replete with all the stages of spermatogenesis and sperm.

cells exhibit complex surface configuration with several pits of various sizes and secretory droplets, mostly seen towards free end (De & Maiti 1987c).

Leydig Cells

Distinct Leydig cells are found in the interstitium between the tubules amidst connective tissue, blood and lymph spaces. Exceptions to this are lizards, *Agama tuberculata* and *Lygosoma himalyanum* from Kashmir. The Leydig cells in A. *tuberculata* are concentrated beneath tunica albuginea like a circumtesticular tunic (Duda & Kaul 1976) and they are reported to be absent (!!) in *L. himalayanum* (Kaul & Duda 1976). The Leydig cells in the lizards, *C. versicolor, Hemidactylus flaviviridis, Chamaeleon calcaratus, P. dorsalis, Mabuya carinata,* and *Varanus monitor* (Gouder & Nadkarni 1974a, 1976a, Shivanandappa & Sarkar 1977a, b, Shivkumar et al. 1979, Sarkar & Shivanandappa 1989), snakes, *Naja naja* and *Python molurus,* the crocodile, *C. porosus* (Gouder & Nadkarni 1974a, b), and *L. p. punctata* (De & Maiti, 1987b) possess enzymes involved in steroid hormone biosynthesis. De and Maiti (1987c) identified three forms (A, B and C) of Leydig cells in *L. p. punctata* based on SEM study. The cell surface showed numerous secretary droplets in form A, and 'crater-like' pits and a few secretory granules in form B. The form C cells are relatively small and shrunken with a few pits on the cell surface. The three forms A, B, and C might indicate different phases of secretory activity of the Leydig cells viz. secretion, exhaustion, and regression respectively.

Testicular Cycles

Spermatogenetic Cycles

Most reptiles exhibit seasonal changes in spermatogenetic activity. The reptilian species, mainly the lizards studied from India exhibit *prenuptial* type of spermatogenesis. In this type, sperm are produced immediately before or during mating season and there exists a synchrony between ovarian and spermatogenetic cycles. The spermatogenetic cycle studied in a few species of lizards, a snake and pond turtles (table 1) is generally distinguished into *recrudescence/ prebreeding, breeding* and *post breeding* phases. The onset of active proliferation of spermatogonia marks the beginning of the recrudescence phase (figure 1B). This is followed by the formation of primary/secondary spermatocytes and spermatids. During breeding phase, testes reach their maximum size with enlarged seminiferous tubules, highly populated with various spermatogenetic stages, and sperm (figure 1C). The Leydig cells are well developed but appear sparse as they are sandwiched between the walls of enlarged seminiferous tubules. Following breeding, the

Table 1 *Annual Testicular cycle of some Indian reptiles*

Species	Breeding season	Locality	References
Hemidactylus flaviviridis	Mar-May	Delhi	Sanyal & Prasad 1967
Hemidactylus brooki	Sept-June	Dharwad	Shanbhag et al. 2000a
Calotes versicolor	Apr-June Apr-Sept	Varanasi Pondicherry Dharwad	Choubey 1970 Kasinathan & Basu 1973 Gouder & Nadkarni 1979
Calotes nemericola	Apr-Sept	Tirupati	Subba Rao & Raja Bai 1972
Psammophilus dorsalis	Apr-Aug	Mysore	Sarkar & Shivanandappa 1989
Sitana ponticeriana	Apr-Sept	Tirupati	Subba Rao & Rajabai 1972
Mabuya carinata	Oct-Dec	Mysore	Sarkar & Shivanaadappa 1989
Natrix piscator	Sept-Nov	Varanasi	Srivastava & Thapliyal 1965
*Naja naja**	Aug	Dharwad	Gouder & Nadkarni 1974a
Lissemys punctata granosa	July-Aug	Varanasi	Singh 1974
Lissemys punctata punctata	May	Calcutta	De & Maiti 1987b

**Season testicular cycle is not studied*

testes begin regression and the individuals enter the post- breeding phase. During the early part of post breeding phase, the seminiferous tubules exhibit residual sperm in the lumen and some germ cells in various stages of degeneration. Subsequently, in the later part of this phase, the seminiferous tubules become shrunken and contain only a few spermatogonia and Sertoli cells (figure 1A).

Division of the testicular cycle into different phases is somewhat arbitrary in species with extended breeding season. An asynchrony in gonadal development among members results into an overlap of different reproductive phases in individuals of a population. The onset of recrudescence, length of testicular activity, and degree and duration of testicular regression also vary among species (figure 2) and even in the same species depending upon its geographic distribution. For instance, *C. versicolor* shows drastic regression of the testes following breeding at Dharwad (Gouder & Nadkarni 1979) and Mysore (Sarkar & Shivanandappa 1989), while testes are spermatogenetically active throughout the year at Pondicherry except that sperm production is low between January-March and June-July (Kasinathan & Basu 1973). Of the two species of geckos studied, *H. flaviviridis* in North India is spermatogenetically active from March-May (breeding season and summer) and following breeding, the testes regress dramatically in size and do not contain advanced stages of germ cells (Sanyal & Prasad 1967). In contrast, in south India, the testes of *Hemidactylus brooki*, are spermatogenetically active almost throughout the year except that the lizards with abundant sperm are very few or absent (figure 3) between July-September, the wet seasons (Shanbhag et al. 2000a). A relatively short testicular activity in *H. flaviviridis* might be due to greater seasonal fluctuations in temperature in north India when compared to that in south India. Apparently, the local climate has a superseding effect over the genetic background in deciding the fine-tuning of the pattern of testicular activity in the lizards.

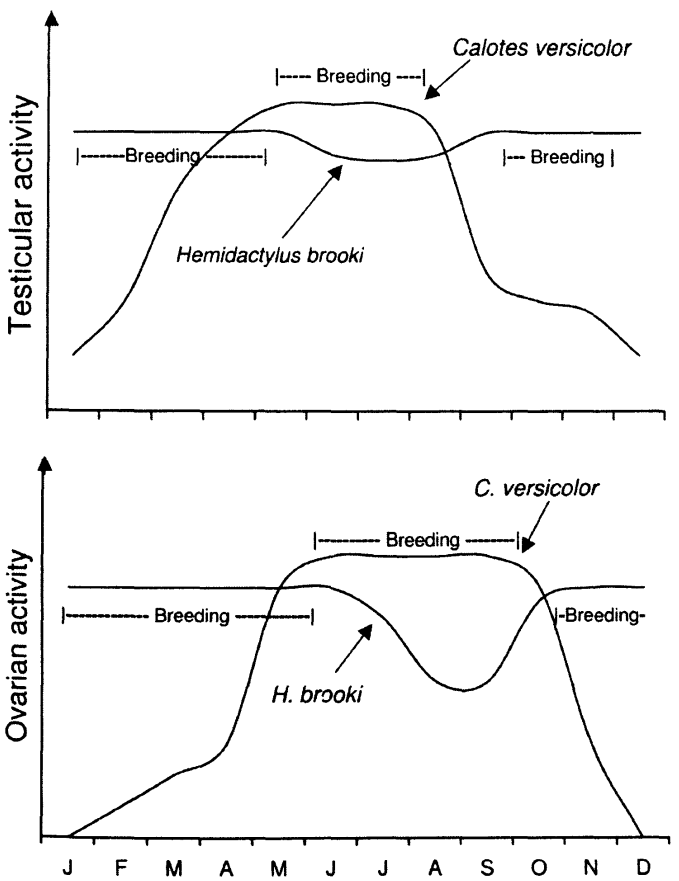

Figure 2 The pattern of annual gonadal activities in *C.versicolor* and *H. brooki*.

Leydig Cell and Androgen Cycles

Seasonal changes in 3β-HSDH activity and lipids associated with cytomorphological changes in the Leydig cells are reported in *C. versicolor*, *P. dorsalis* and *M. carinata* (Shivanandappa & Sarkar 1978,1979, Gouder & Nadkarni 1979, Shivakumar et al. 1979). The enzyme activity is high in the Leydig cells in the breeding season when testes are spermatogenetically active. The correlative seasonal changes in the plasma T levels and testicular activity are studied in *C. versicolor* (Radder et al. 2001). The plasma *T* levels vary significantly with testicular mass (figure 4). Lowest plasma T levels (0.55 ± 0.03 ng/ml) are observed in December, the postbreeding quiescent phase. With the onset of recrudescence in February, plasma T levels increase slightly (0.86± 0.18 ng/ml). A further rise in plasma T (6.15 ± 1.53ng/ml) seen in April is correlated with the advancement in spermatogenesis. In June however, plasma T level (12.92 ± 1.19 ng/ml) reaches its peak in association with the greatest testicular activity, and breeding. After breeding in October, plasma T levels decline (2.02 ± 1.22ng/ml). In general, plasma T levels are highest in individuals with greatest spermatogenetic activity (figure 5).

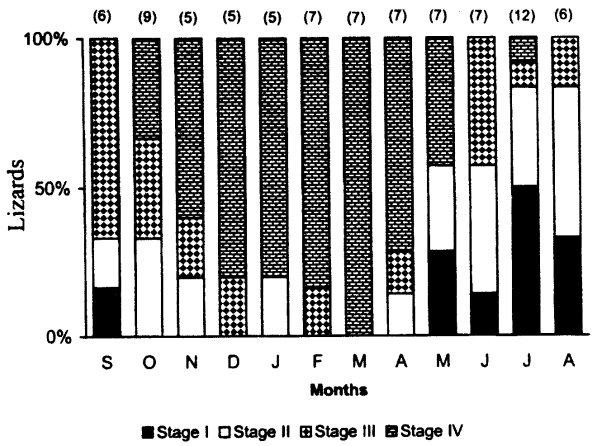

Figure 3 The percentage of lizards (*H. brooki*) showing stage I to stage IV testicular activity in monthly sample around the year. Stage I: with spermatogonia and primary and spermatocytes, Stage II: all stages of spermatogenesis with a few spermatids and sperm, Stage III: all stages of spermatogenesis with moderate quantity of spermatids and sperm and Stage IV: all stages of spermatogenesis with abundant spermatids and sperm. (from Shanbhag et al. 2000a)

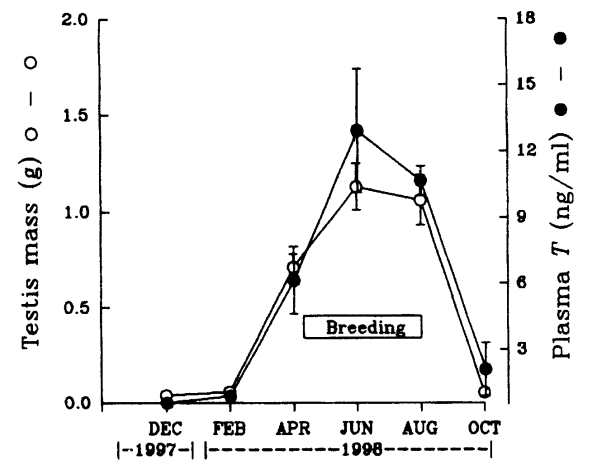

Figure 4 Changes (mean ± SE) in plasma T and testicular mass during reproductive cycle in *C. versicolor* (from Radder et al. 2001)

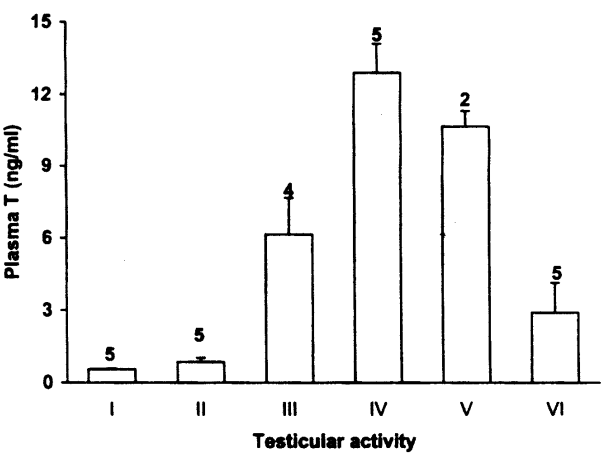

Figure 5 Changes in plasma T (Mean ± SE) and testicular activity (spermatogenesis) in *C. versicolor*. Number above bars indicates sample size. (From Radder et al. 2001)

Male Accessory Reproductive Organs

Epididymis and Vas Deferens

The Wolffian duct in reptiles gives rise to vas deferens. The genital region of Wolffian duct differentiates into epididymis. The latter is a simple structure composed of convoluted tubule of varying diameter along its length lined by a layer of epithelial cells. Epididymal structure is described in detail in *H. flaviviridis* (Haider & Rai 1987) and *C. versicolor* (Averal et al. 1992). In reproductively active lizards the epithelial cells in the anterior part of the epididymial tubule exhibit secretory granules. The epididymal tubule in the posterior region is the largest in diameter and is lined by columnar cells with short stereocilia. This region becomes densely filled with sperm during breeding season (figure 6A). Proteins, lipids and various steroid dehydrogenases and hydrolases have been localized in the epididymis of *C. versicolor* and *M carinata*, *P. dorsalis* and *H. flaviviridis* (Shivkumar et al. 1979, Shivanandappa & Sarkar 1986). The ultrastructural studies on the epididymis of *C. versicolor* and *M. carinata* have shown the presence of well developed Golgi complex, endoplasmic reticulum and secretory granules in the epithelial cells (Sarkar & Shivanandappa 1989). The epididymal fluid contains glycerophosphorylcholine, sialic, pyruvic and lactic acids but no glucose or fructose in *H. flaviviridis* (Nirmal & Rai 1999). The protein content is high in posterior part of epididymis especially during spermatogenetically active phase (Nirmal & Rai 2000). The fluid from posterior epididymis in the lizard is more potent in influencing sperm motility; its high sialic acid content might be one of the factors responsible for sperm maturation (Nirmal & Rai 1997, 1999).

The epididymis of lizards shows marked changes in its size that is correlated with the Leydig cell activity. During quiescent phase, the epithelial cells appear small and cuboidal with hyperchromatic nuclei (figure 6B). Experimental studies involving castration and androgen replacement therapy have shown androgen dependency of epididymis in lizards (Akbarsha & Balsubramanian 1982, Sarkar & Shivanandappa 1984, Haider 1985a, Shivanandappa & Sarkar 1987). The treatment with antiandrogens such as cyproterone acetate (CPA) and flutamide caused

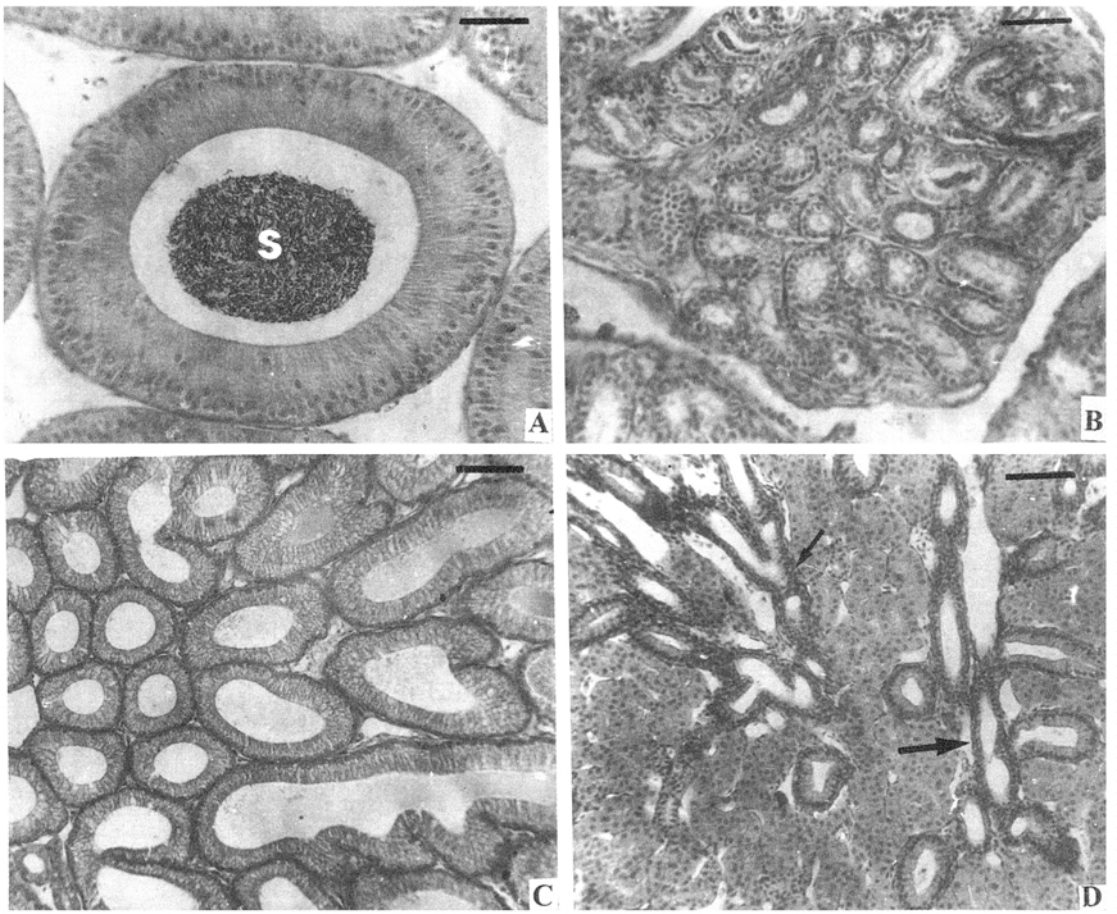

Figure 6 A-B Sections of epididymis, and C-D: sections of kidney passing through renal sexual segment of *C. versicolor*; A, Epididymis during breeding phase. The epididymal tubule are large and lined with tall epithelial cells. Note the presence of sperm in its lumen; B, Epididymis during post-breeding phase. Note the small epididymal tubules lined with low epithelial cells; C, Renal sexual segment during breeding season. Note the enlarged tubules; D, Renal sexual segment during post-breeding season. Note that tubules are regressed (arrows)

regression of epididymis in *P. dorsalis* (Shivakumar & Sarkar 1980) and *H. flaviviridis* (Haider & Rai 1986). Similarly, flutamide inhibited FSH or T induced growth of epididymis in *H. flaviviridis* (Rai & Haider 1991). These studies indicate that androgens are needed for normal epididymal growth and activity in lizards.

The vas deference in lizards is a simple convoluted tube carrying the sperm from epididymis. Anatomy of vas deferens is described for *C. versicolor*. It has an outer layer of smooth muscles and has luminar trabeculae with psuedostratified epithelium. During breeding season the terminal part of vas deferens appears swollen and is comparable to the ampulla of mammalian vas deferens (Akabarsha & Meeran 1995). During the quiescent phase, the vas deferens can not be distinguished into ductal and ampullary parts. However, administration of T during quiescent phase, the ampullary region becomes distinct and glandular suggesting its androgen dependency (Akbarsha & Meeran 1995).

Renal Sexual Segment

The renal sexual segment (RSS) in male lizards and snakes originates from nephrons, generally in the caudal end of the kidney. In most squamates, the renal sexual segment includes mostly distal convoluted tubules and in some, the collecting tubules form its main part (Fox 1977). The RSS is studied in *H. flaviviridis, C. versicolor, P. dorsalis* and *M. carinata* (Sanyal & Prasad 1966, Sarkar & Shivanandappa 1989). In all these species, tubules of the RSS undergo hypertrophy and become secretory during the breeding season and involute in non-breeding season (figure 6C & D).

The secretion of RSS that contains phospholipids, proteins and amino acids is known to be androgen dependent in *H. flaviviridis, C. versicolor* and *M. carinata* (Sanyal & Prasad 1966, Reddy et al. 1972, Sarkar & Shivanandappa 1989). The secretory granules are hydrophobic in nature and mainly made up of peptides containing high amounts of tyrosine, tryptophan and disulphide residues (Sarkar & Shivanandappa 1984). The enzyme acid phosphatase is localized in RSS in all these lizard species (Deb & Sarkar 1963, Sanyal & Prasad 1966, Sarkar & Shivanandappa 1984) and is also shown to be androgen dependent (Prasad & Sanyal 1969, Reddy & Prasad 1970a, Sarkar & Shivanandappa 1984). An association between acid phosphatase and phospholipids from RSS with esterase in cloaca and the reproductive tract of the female, may lead to enzymatic degradation of phospholipids yielding the liberation of fatty acids and glycerophosphorylcholine (Sanyal & Prasad 1966). These may serve as a source of energy, a substrate for survival of spermatozoa in the oviduct. Prasad and Reddy (1972) suggested a homology of the reptilian RSS with the mammalian seminal vesicle since both share certain common features like embryological origin, relationship with vas deferens, and response to androgen. Further, the secretion of RSS in lizards may constitute a "primitive semen"- a nutrient and transporting medium for sperm and also to prevent their desiccation (Sarkar & Shivanandappa 1989).

Endocrine Regulation of Testicular Cycle

Our knowledge about the endocrine regulation of testicular activity is mostly derived from studies on temperate lizards and snakes. The parietal-pineal complex through secretion of melatonin is believed to play a role in controlling seasonal reproduction of lizards (Duvall et al. 1982, Licht 1984). Among the Indian lizards, in *C. versicolor*, pinealectomy accelerated recrudescence and delayed regression of testes (Haldar & Thapliyal 1977, Thapliyal & Haldar 1979). However, the experiments were not controlled for temperature, an important proximate factor controlling testicular cycles in poikilotherms and therefore no definite conclusions can be drawn from these studies.

Specific brain areas like the hypothalamus and preoptic areas play a role in the regulation of testicular cycle. In the turtle, GnRH is known to induce release of gonadotropins (GtH) from pituitary *in vitro* (ref. Duvall et al. 1982, Licht 1984, Whittier & Crews 1987). A recent study on *C. versicolor* has shown that GnRH can induce testicular recrudescence even during the post-breeding quiescent phase suggesting, *albeit* indirectly, hypothalamic regulation of pituitary GtH *vis-a-vis* reproduction (Shanbhag et al. 2000b).

Work on biological and chemical properties of reptilian GtH was carried out by Licht and his collaborators in 1970s (Ishii 1991). They postulated that in squamates only one GtH exists that is chemically similar to mammalian FSH. On the other hand, the pituitaries of turtles and crocodiles contain two types of GtH that correspond chemically to mammalian FSH and LH respectively (Licht 1984). Purified or isolated reptilian GtH are not commercially available. The research on gonadal regulation involving pituitary hormones in reptiles is therefore exclusively based on the use of mammalian GtH.

In *N. naja* seasonal variation in plasma GtH is correlated with testicular activity (Licht 1984). Corresponding studies are lacking for reptiles inhabiting India. Studies involving unilateral castration (ULC) in *C. versicolor* indirectly suggest low levels of GtH during postbreeding season and a rise during recrudescent phase (Sharma & Shanbhag 1991). ULC induces compensatory hypertrophy of the contralateral testis during recrudescent phase and not during postbreeding phase. Studies to elucidate the role of GtH in the regulation of spermatogenesis and Leydig cell function in lizards are inconsistent and confusing. Hypophysectomy caused regression of testes during recrudescence phase in both *H. flaviviridis* (Reddy & Prasad 1970b) and *C. versicolor* (Gaitonde & Gouder 1981). A decrease in 3β-HSDH, 17β-HSDH and G-6-PDH enzyme activities in the Leydig cells, regression of epididymis and RSS in the hypophysectomised lizards also implies lower output of androgens (Reddy & Prasad 1970b, Gaitonde & Gouder 1981). The mammalian FSH or PMSG could stimulate spermatogenesis in hypophysectomised as well as intact *H. flaviviridis* during regression phase (Reddy

& Prasad 1970b, Rai & Haider 1986), while LH, HCG and testosterone failed to stimulate testes. Simulation of the RSS following FSH or PMSG treatment in *H. flaviviridis* suggests that a mammalian FSH-like hormone functions as gametogenic as well as steroidogenic hormone. In contrast, both ovine FSH and LH stimulated spermatogenesis as well as Leydig cell activity in *C. versicolor* (Gaitonde & Gouder 1985) during both regression and recrudescence phases. Two types of basophils, B_2 and B_3 associated with FSH and LH secretion were tinctorially identified based on their response to gonadectomy, administration of methallibure and testosterone in *C. versicolor* and *H. flaviviridis* (Mohanty & Naik 1984). The comparative studies on species belonging to different families are needed to know the types of GtH produced by phylogenetically distant taxa among squamates.

In mammals, it is known that LH stimulates Leydig cells' androgen output that in turn regulates meiotic division of germ cells (Callard 1991). Among the Indian reptiles testosterone propionate (TP) is reported to induce spermatogenesis and sperm production in the lizard, *Uromastix hardwickii* and immature *Crocodylus palustris* (Ramaswami & Jacob 1963, 1965). However, T and T +FSH when injected to mature *H. flaviviridis* having regressed testes did not have any effect on spermatogenesis (Reddy & Prasad 1970b, Rai & Haider 1986). A few studies carried out on lizards attempt to elucidate the role of T in spermatogenesis by using antiandrogens. The results vary. In *C. versicolor*, injection of low doses of cyproterone acetate (CPA) during breeding period did not affect the spermatogenesis though it caused regression of epididymis. In contrast, high doses of CPA arrested formations of spermatids (Gaitonde & Gouder 1983). They opined that high doses of CPA might interfere with hypothalamic release of GtH resulting in the arrest of spermatogenesis. Rai and Haider (1991) studied the effect of FSH, flutamide (non- steroidal antiandrogen known to block negative feedback of T on hypothalamo-hypophysial axis and promote release of more gonadotropins), FSH + flutamide and T on recrudescing testes in *H. flaviviridis*. FSH alone or FSH + flutamide could induce complete spermatogenesis. However, flutamide treatment, despite stimulation of Leydig cell androgen production, did not induce spermatogenesis. Exogenously administered T inhibited spermatogenesis. Thus, it appears that FSH alone (or FSH-like protein) regulates mitotic and meiotic divisions, spermiogenesis and also Leydig cell activity in *H. flaviviridis*. In a similar experiment, treatment with FSH, T, and CPA alone, or in combination with FSH or T on the recrudescing male *H. flaviviridis* also showed that onset of spermatogenetic activity require FSH (or FSH-like protein) and not androgen (Rai & Haider 1995).

Other endocrine glands also play a role in testicular cycles. The studies on thyroid- testis interaction among Indian reptiles are few. Thyroidectomy caused testicular regression in *C. versicolor*. Thyroxine injection (induced hyperthyroidism) also caused testicular regression. However, the effect of hypo- or hyper thyroidism varies seasonally, being greater when testes are enlarged during recrudescent phase (Haldar-Mishra & Thapliyal 1981). In thyroidectomized snakes, *N. piscator* the testis weight was reduced at higher temperature than at lower temperature (Thapliyal et al. 1974). These studies indicate that extreme perturbation in thyroxine levels leads to deleterious effects on testicular activity. More studies are needed in different species to elucidate the exact role of thyroid hormones in testicular regulation.

Correlative seasonal changes in the adrenocortical cells and gonads have been reported in several reptilian species (Lofts 1978) A seasonal variation in the adrenal gland activity is correlated with testicular cycle in *C. versicolor* and *M. carinata*. The adrenal activity is highest during breeding phase in the lizards (Kasinathan & Basu 1973, Chadramohan & Yajurvedi 1995-96). In adrenalectomised *M. carinata* testicular recrudescence and maintenance were severely impaired (Yajurvedi & Chadramohan 1993), while administration of either dexamethasone or LH + FSH to adrenalectomised skinks during recrudescence phase resulted in the restoration of testicular activity (Yajurvedi & Chadramohan 1994). However, higher dose (40μg) of corticosterone inhibited normal and FSH-

induced testicular recrudescence and rise in plasma T levels in the skink (Yajurvedi & Nijagal 2000). The results indicate that high levels of corticosterone inhibit FSH-induced testicular recrudescence, possibly by suppressing testosterone secretion. The above findings indicate that corticosteroids affect testicular functions in lizards.

The Female Reproduction

Morphology of the Ovary

All reptiles possess a pair of ovaries that are generally symmetrical and covered by a layer of flattened epithelial cells. Like in most vertebrates, the ovaries of reptiles exhibit various developmental and degenerative processes depending on the stage of development and/or the phase of ovarian cycle. These include the processes like oogonial proliferation, oogenesis, follicular growth, vitellogenesis, ovulation, luteogenesis, luteolysis and follicular degeneration. Oogonial proliferation and oogenesis occur throughout the life. *Sphenodon punctatus* is an exception in which oggenesis is restricted to embryonic stages (ref. Franchi et al. 1962).

Germinal Bed

The oogonia and oocytes are restricted to one or more discrete regions in the ovary known as germinal beds (GBs). The number of GBs varies among the species. Each ovary contains one GB (figure 7 A& B) in monoautochronic lizards such as *H. flaviviridis* (Guraya & Verma 1976) and *H. brooki* (Shanbhag et al. 1998). The polyautochronic lizard, *C. versicolor* has two GBs (Figure 7 C & D) in each ovary (Jones *et al.* 1982, Shanbhag & Prasad 1993a) and not one as reported by Verma (1970) and Sarkar and Shivanandappa (1989). The ovary of *Sitana ponticeriana* also contains two GBs (B A Shanbhag, et al. unpublished observations). Though *P. dorsalis* and *M. carinata* are polyautochronic lizards only one GB is reported in each ovary (Sarkar & Shivanandappa 1989). It is believed that the number of GBs might have a role in controlling the clutch size in reptiles (Jones et al. 1982).

A quantitative estimation of oogonia and oocytes in the GB is carried out only in two species of Indian lizards, *C. versicolor* and *H. brooki*. The two species exhibit different clutch patterns. For instance, *C. versicolor* has a variable clutch size with many eggs whereas *H. brooki* has fixed clutch size

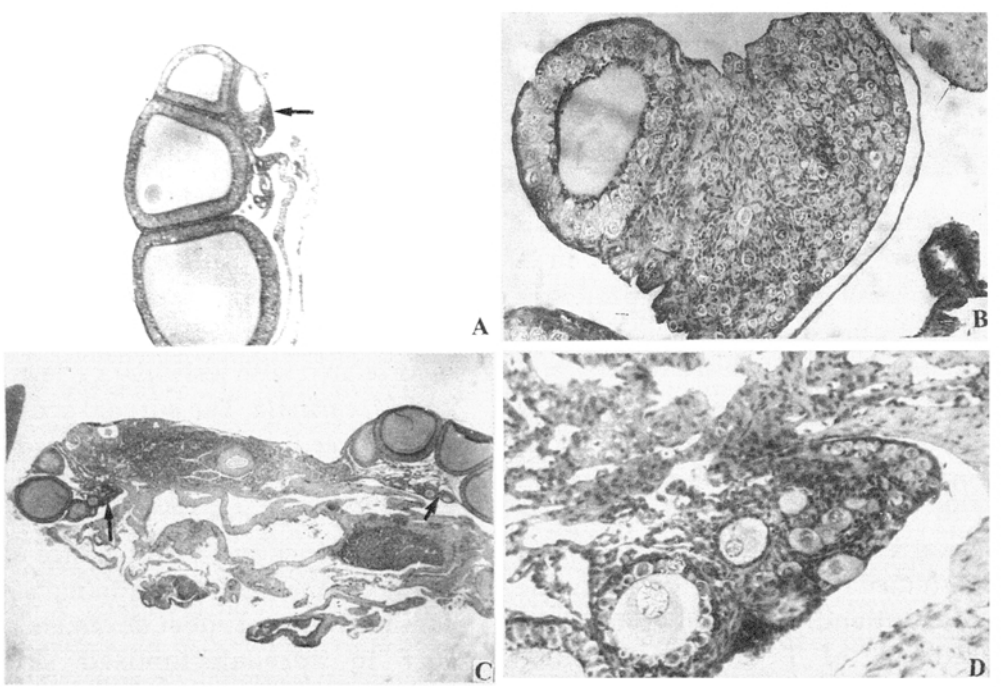

Figure 7 A-B and C-D are sections of the ovary passing through germinal bed of *H. brooki* and *C. versicolor* respectively A, Shows the organization of follicles and a single germinal bed (arrow); B, The magnified view of germinal bed (figure A) containing oogonia and oocytes; C, Shows the bilobed nature of the ovary and two germinal beds (arrows); D, The magnified view of germinal bed (figure C).

of 2 eggs. In *C. versicolor*, the GB contains a large number of oogonia (642-696) during early vitellogenic to early breeding phase (April-June) while the oggonial number is low in other phases of the ovarian cycle (Shanbhag & Prasad 1993a). In contrast, in *H. brooki* no significant variation in the number of oogonia and oocytes is encountered during different phases of the ovarian cycle suggesting uniform germinal bed activity throughout the ovarian cycle (Shanbhag et al. 1998).

Follicular Structure

In the ovary of reptiles, investment of an oocyte by prefollicular cells derived from germinal epithelium begins before or at the onset of diplotene stage (Tokarz 1978, Guraya 1989, 1998). With the investment by prefollicular cells, the oocytes move inwards from the GB. The oocyte becomes completely invested by a single layer of flattened epithelial cells to form primordial follicle. As the ovarian follicle grows it exhibits three basic cellular components, the oocyte, granulosa and theca. In turtles, the granulosa consists of a single layer of cuboidal or columnar epithelium throughout the oocyte growth. In contrast, granulosa exhibits complexity in squamates, amphisbaenians and tautara. It initially becomes bilayered and then multilayered after a follicle leaves the GB (Verma 1970, Verma & Kumar 1987, Guraya 1989, 1998, Shanbhag et al. 1998). The granulosa layer exhibits three types of cells viz. small, intermediate and flask-shaped pyriform cells (figure 8A). The small cells are further distinguished into apical and basal cells depending on their position in the granulosa layer. The flask shaped pyriform cells show narrow protoplasmic protrusions that traverse through the zona pellucida. Hence, the cytoplasm of oocyte and pyriform cells become confluent with each other (Verma 1970, Guraya & Verma 1976). Factors regulating the intercellular bridges of communication remain to be determined.

With the growth of the previtellogenic oocyte, a number of nuclear and cytoplasmic changes take place including the formation of a juxtanuclear Balbiani vitelline body (Guraya 1989). Balbiani body of *H. flavivirdis* consists of a basophilic yolk nucleus (composed of protein and RNA), mitochondria, Golgi bodies and lipid droplets (Guraya 1968). After attaining full development and differentiation during oocyte growth the Balbiani body disintegrates and its inclusions disperse throughout ooplasm especially in its cortical zone (Saxena 1979). Possibly, the cortical cytoplasm forms an active metabolic site in large previtellogenic oocytes. The occurrence of yolk nucleus substance (Golgi, mitochondria and ER) in the peripheral cytoplasm may be related to the process of absorption, digestion and utilization of various substances from follicular cells (Guraya 1989).

With the onset of vitellogenesis the pyriform cells start disintegration (figure 8B). Some bag-like structures which consist of follicle cells and zona pellucida projecting from follicular epithelium into the oocyte is reported in *H. flaviviridis* (Guraya & Verma 1976). The significance of such structures is unknown. In vitellogenic follicles, the granulosa becomes single layered (figure 8C) and monomorphic (Guraya 1989, Shanbhag et al. 1998). Various hydroxysteroid dehydrogenases involved in steroid biosynthesis are reported in the follicular wall of *C. versicolor, H. flaviviridis, C. calcaratus* (Gouder & Nadkarni 1976b), *Varanus monitor* and *P. dorsalis* (Sarkar & Shivanandappa 1989). A weak enzyme activity in the granulosa and a moderate activity in some cells of the theca interna was reported in *C. versicolor, H. flaviviridis, C. calcaratus* (Gouder & Nadkarni 1976b), while the enzyme activities were intense in cells of the theca interna in *V. monitor* and *P. dorsalis* (Sarkar & Shivanandappa 1989). No steroidogenic enzymes were found in the theca of *M. carinata* and *M. trivitata* (Sarkar & Shivanandappa 1989). The ooplasm in the center and periphery of developing and vitellogenic follicles respectively in *C. versicolor, H. flaviviridis, C. calcarata, P. dorsalis* and *Lacerta sicula* also exhibited weak steroidogenic enzyme activities (Gouder & Nadkarni 1976b, Saxena 1977, Sarkar & Shivanandappa 1989). The significance of the steroidogenic enzymes in the ooplasm is unknown.

Follicular Atresia

Like in other vertebrates, atresia may occur at any stage of follicular development in the ovaries of reptiles (Saidapur 1978, Guraya 1989). Ultrastructural details of follicular atresia are not known in reptiles.

The histology of previtellogenic and vitellogenic follicles undergoing atresia is described in Indian lizards like *C. versicolor* (Verma 1970, Verma and Guraya 1973a, Gouder et al. 1979), *H. flaviviridis* (Guraya & Verma 1976), *M. carinata*, *P. dorsalis* (Sarkar & Shivanandappa 1989) and a snake, *Bungarus coeruleusi* (Guraya 1965). In general, atresia of previtellogenic follicles is more extensive while that of vitellogenic follicles is rare (Verma 1970, Shanbhag & Prasad 1993a). The presence of steroid dehydrogenases and lipids is shown in the follicular cells of degenerating follicles in lizards and snakes (Gouder & Nadkarni 1976b, Guraya & Verma 1976, 1978, Gouder et al. 1979). The enzyme activity and lipid droplets disappear from granulosa cells as the atresia advances. However, some thecal cells persist within the stroma and exhibit steroid dehydrogenase activities (Guraya 1989). These cells are believed to contribute to the interstitial gland cells of the ovary (Saidapur 1978). Exact mechanisms and the factors causing follicular atresia are not fully understood. Since a larger number of follicles are recruited than those that ultimately ovulate suggests that there is some intraovarian mechanism determining the follicles destined to ovulate. Several physiological and ecological factors are known to affect follicular development. Physiological factors like inadequate levels of GtH, steroid hormones, blood supply to the ovary and nutrition are the possible candidates causing follicular atresia. Ecological factors like inadequate light, temperature, captivity, crowding etc are also reported to cause follicular atresia. These latter factors may cause some sort of physiological stress (Saidapur 1978, Guraya 1989).

Corpus Luteum

Most reptiles whether oviparous or viviparous develop secretory corpora lutea (CL) following ovulation (Saidapur 1982, Xavier 1987, Guraya 1989). The histological changes that occur during luteogenesis and luteolysis are described in *M. carinata* (Annamalai 1966), *C. versicolor* (Verma & Guraya 1973b, Shanbhag et al. 2001), *H. flaviviridis* (Guraya & Verma, 1976), *P. dorsalis* (ref. In Sarkar & Shivanandappa 1989), *H. brooki* (Shanbhag et al. 1998) and in the snake, *Lycodon aulicus* (Guraya 1973). In majority of reptiles studied so far, the CL is formed mainly from the granulosa cell. Following

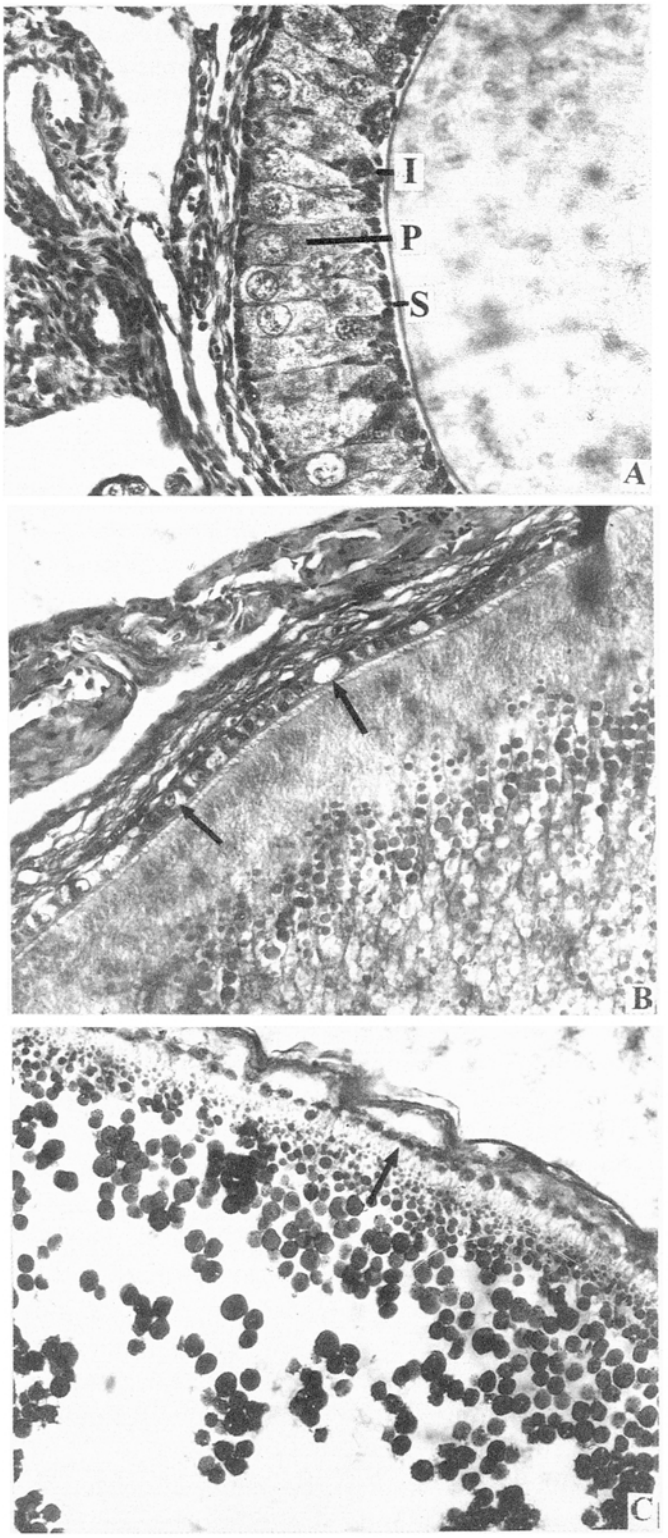

Figure 8 A-C Sections of the ovary of *H. brooki*; A, A part of previtellogenic follicle with polymorphic granulosa; P- pyriform, I- intermediate and S- small cells; B, A part of early vitellogenic follicle with pyriform cells (arrows) undergoing disintegration; C, A part of vitellogenic follicle. Note that the granulosa is made up of a single layer of only small cells (arrow).

ovulation, the granulosa cells hypertrophy and form luteal cells. Due to contraction and shrinkage of the postovulatory follicle, the luteal cell mass occupy the follicular lumen evacuated by the ovum. The thecal layers also undergo well-defined morphological changes. The theca interna mainly consists of hypertrophied round cells with clear cytoplasm, fibroblasts, collagen fibers and macrophages. Theca externa is thin and more fibrous than cellular. Fully developed CL forms a compact structure with central lumen filled with granulosa luein cells with thecal layers as supporting tissue. In *M. carinata, H. flaviviridis* and *H. brooki,* the fibroblasts and blood capillaries do not invade the central luteal mass (Guraya & Verma 1976, Sarkar & Shivanandappa 1989, Shanbhag et al. 1998), while in *L. silicus, C. versicolor* and *P. dorsalis,* the luteal mass is interspersed with fibrous septa and blood capillaries during later stages (Guraya 1973, Verma, 1970, Sarkar & Shivanandappa 1989). There are relatively a few studies on the ultrastructural aspects of CL in reptiles and none on Indian species (Saidapur 1982, Xavier 1987).

Both oviparous lizards and snakes are known to retain eggs in the oviduct for species specific time before oviposition. In gravid lizards and snakes, CL exhibit 3β-HSDH activity and secrete progesterone (P) (Saidapur 1982, Xavier 1987). In squamates the plasma P is reported to peak shortly after ovulation or at mid- or even late gestation depending on the species (Xavier 1987). In *C. versicolor*, 3β-HSDH was reported in the granulosa lutein cells, but the stage of gestation was not recorded (Gouder & Nadkarni 1976b). With the degeneration of CL, sudanophilic lipids accumulate in the luteal cells in both *C. versicolor* (Verma & Guraya 1973b) and *H. flaviviridis* (Guraya & Verma 1978).

Recently, temporal relationship between the stage of embryonic development, luteal 3β-HSDH activity and plasma P levels has been studied in *C. versicolor* (Shanbhag et al. 2001). The study shows that 3β-HSDH activity in granulosa lutein cells was weak immediately following ovulation (eggs in the oviduct but without shell), but increased during eggshell formation (figure 9A). Subsequently, the enzyme activity decreased with the advancement in the age of the oviductal embryos (figure 9B) and only a trace activity was seen from mid gestation (stage 16-18 embryo) to

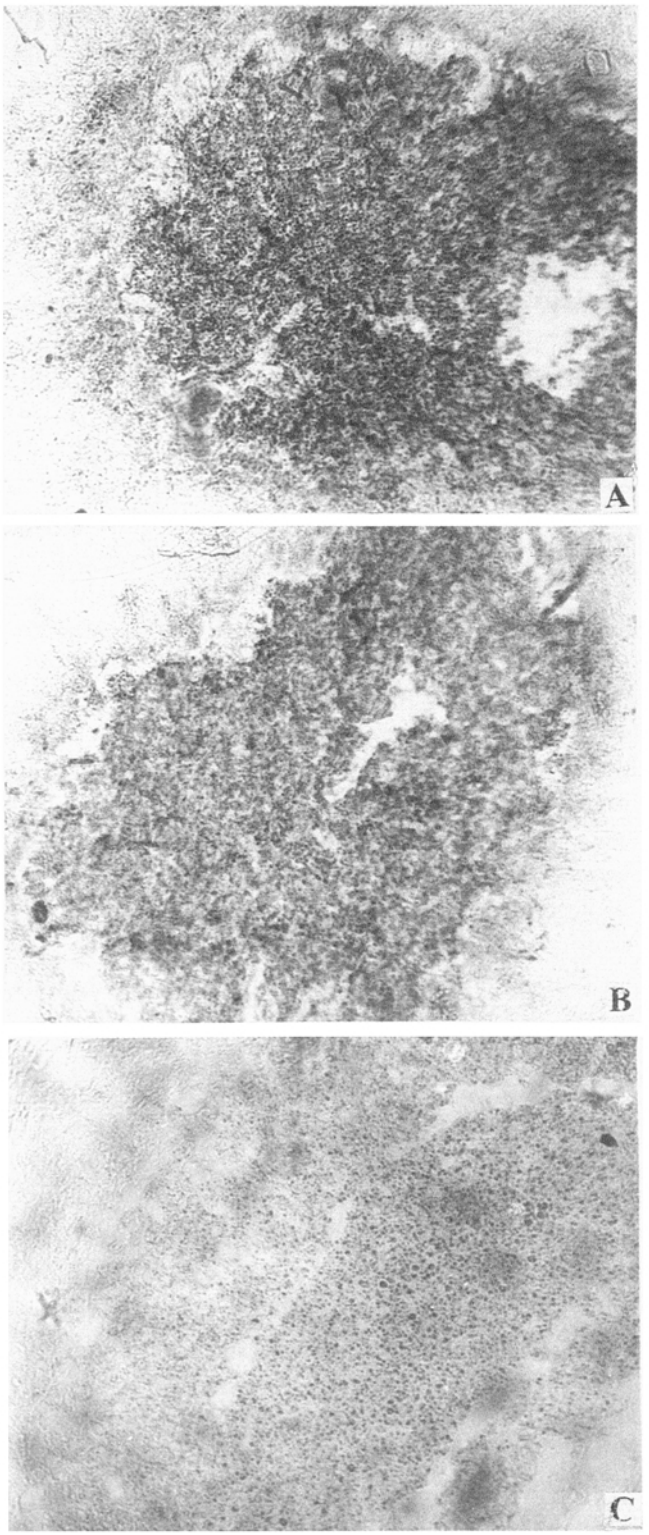

Figure 9 A-C Fresh frozen section of CL of *C. versicolor* showing 3β-HSDH activity in the granulosa cells during early gestation, mid gestation and prior to ovipositon respectively. Note an intense 3β-HSDH activity in A, a decrease in enzyme activity in B and a trace enzyme activity in C.

oviposition (figure 9 C). Plasma P levels also showed a similar pattern paralleling changes in luteal 3β-HSDH activity being highest during eggshell formation (stage II) and lowest following mid-gestation (stage IV) (figure 10). These findings suggest that elevated levels of plasma P are not essential after mid-gestation in the lizard. Also in *M. carinata* lutectomy on days 5 to 8 of gestation had no effect on the duration of gestation and development of the embryo in the oviduct (Sekharappa & Sarkar 1978) thereby negating the role of CL in the later part of gestation.

Calotes versicolor normally retains eggs in the oviduct for about 2 weeks following ovulation (Shanbhag & Prasad 1993b) and sometimes for prolonged period as long as 180 days if proper oviposition site is not found (Radder et al. 1998). In such cases, the embryonic growth in the oviductal eggs is arrested at stage 34 and the plasma P levels rise. Interestingly, in such cases CL exhibits only a trace 3β-HSDH activity while the enzyme activity (figure11A & B) increases markedly in the adrenocortical cells (Shanbhag et al. 2001). This suggests that adrenal glands rather than CL contribute to a rise in plasma P during prolonged egg retention. Prolonged egg retention is considered to be a step in the evolution of viviparity (Shine & Guillette 1988) that is possibly accomplished by secretion of P and corticosterone by the adrenal under *stressful* condition. The findings on *C. versicolor* clearly indicate that P secretion by the adrenal gland rather than by CL promotes oviductal egg retention and thereby provide experimental support to the "stress induced evolution of viviparity" hypothesis (Shanbhag et al. 2001).

Stroma and Interstitial Gland Cells

The ovarian stroma mainly consists of fibrous connective tissue, degenerating CL and atretic follicles, blood vessels, lymphatics, nerves and interstitial gland cells. The amount of interstitial gland cells varies depending upon the phase of ovarian cycle. The interstitial gland cells in the ovary of *C. versicolor, H. flaviviridis, C. calcaratus* (Gouder & Nadkarni 1976b), *V. monitor, M. carinata, M. trivittata* and *P. dorsalis* (Sarkar & Shivanandappa 1989) exhibit 3β- HSDH activity.

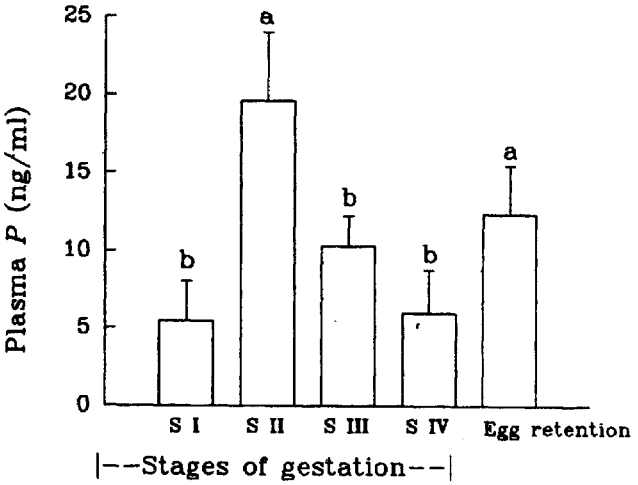

Figure 10 Plasma levels (Mean ± SE) of P during different stages of gestation and prolonged egg retention in *C. versicolor*. Dissimilar alphabets on the bars indicate significant difference while, similar alphabets indicate no significant difference among the groups. (from Shanbhag et al. 2001)

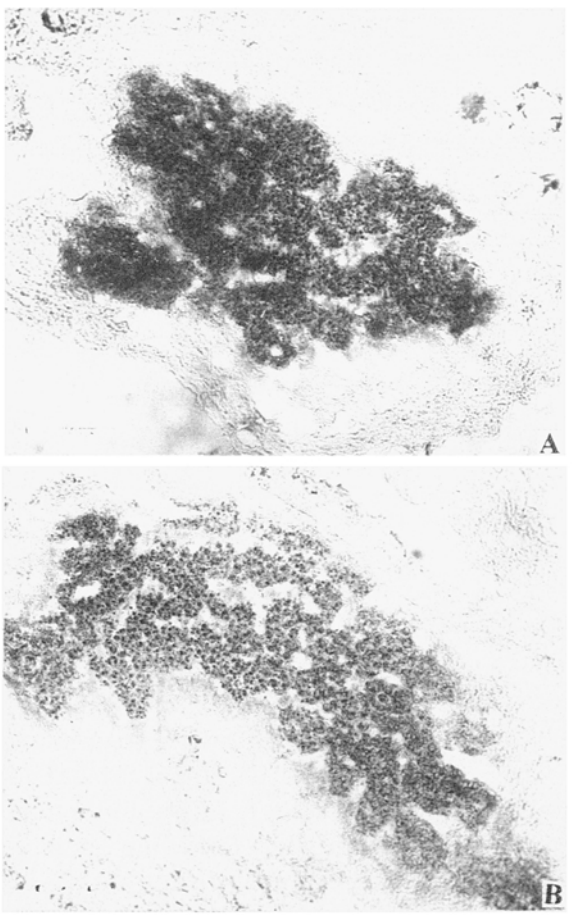

Figure 11 A & B Fresh frozen sections of adrenal gland of *C. versicolor* during early gestation and with prolonged egg retention. Note an intense 3β-HSDH in adrenocortical cells in figure A (egg retained lizards) compared to figure B (early gestation).

Mast Cells

Mast cells in the ovary of mammals and birds are known to play an important role in regulating the permeability of blood capillaries. In *C. versicolor*, distinct granulated mast cells are seen in the theca interna of large previtellogenic follicles (figure 12A). The mast cell number and activity seem to increase with follicular growth. Large vitellogenic follicles exhibit degranulated mast cells suggesting release of secretory material from them. These cells have also been observed in the stroma (figure12B) (Shanbhag & Prasad 1997). Granulated mast cells are observed in the later stages of CL. An increase in stromal mast cells during post-breeding phase suggests that these are probably contributed to stroma by the degenerating CL.

Ovarian Cycles

Ovarian cycles have been studied in a large number of reptiles mainly representing lacertilia, ophidia and chelonia. (Fox 1977, Angilini & Ghiara 1984, Whittier & Crews 1987, Sarkar & Shivanandappa 1989, Vitt 1992). Reptiles inhabiting temperate regions are strictly seasonal breeders while those from tropics exhibit a continuum from acyclic to cyclic breeding (Angilini & Ghiara 1984, Saidapur 1989). The lizards and snakes studied thus far from India exhibit oviparous mode of reproduction and are seasonal breeders, some with extended breeding season (figure 2). But for a lone example of viviparous skink, *Mabuya multifasciata* inhabiting Asam and Nicobar Island (Smith 1935), there are no reports on viviparity among Indian lizards and snakes. The studies on ovarian cycle of Indian reptiles are limited to 6 species of lizards, *C. versicolor, H. Flaviviridis, P. dorsalis, M. carinata, S. ponticeriana, H. brooki* and a snake, *Xenochrophis piscator* and the pond turtles, *Lyssemis punctata granosa and L. p. punctata* (table 2). Based on the ovarian-somatic index and morphological changes, the ovarian cycle in these reptiles can be distinguished into recrudescence or preparatory phase, breeding and postbreeding regression phases.

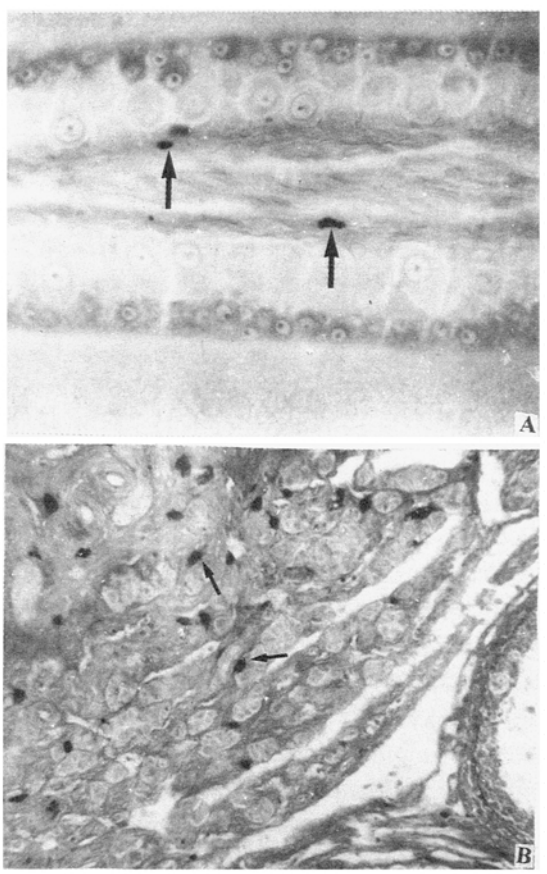

Figure 12 A & B section of ovary of *C. versicolor* stained with toludine blue; A, Mast cells (arrows) in the theca interna of previtellogenic follicles; B, Mast cells in the stroma (arrows)

Only smallest sized previtellogenic follicles, and atretic follicles are found in the ovaries during postbreeding regression phase. Appearance of large previtellogenic follicles lined with polymorphic granulosa marks the onset of recrudescence phase. During this phase, due to vitellogenesis, the size of the follicles increases. During breeding phase, ovulation and mating is followed by gestation of oviductal eggs. Postbreeding regression normally begins after oviposition in all reptiles that reproduce in a single bout. In multiclutched lizards, like *C. versicolor*, during later part of gestation of the first clutch, vitellogenic follicles for the subsequent clutch begin to develop (Shanbhag & Prasad 1993a, Shanbhag et al. 2000c). In such species, the ovarian regression begins only after oviposition of the last clutch of the season. *Calotes versicolor* lays 2-3 clutches per breeding season.

Follicular Kinetics

Studies on the ovarian cycle in reptiles generally describe gross morphology and size of the largest follicles. The quantitative aspect of ovarian follicular dynamics is studied only in four species, *Lepidodactylus lugubris* (Jones et al. 1978),

Table 2 *Annual Ovarian cycle of some Indian reptiles*

Species	Breeding season	Locality	References
Hemidactylus Flaviviridis	Mar-May	Delhi Ludhiana	Sanyal & Prasad 1967 Verma & Guraya1975
Hemidactylus brooki	Sept-June	Dharwad	Shanbhag et al. 1998
Calotes versicolor	June-Sept	Ludhiana	Verma & Guraya 1975
	May-Oct	Dharwad	Shanbhag & Prasad 1993a
Calotes nemericola	Apr-Sept	Tirupati	Subba Rao & Raja Bai 1972
Psammophilus dorsalis	Apr-Aug	Mysore	Sarkar & Shivanandappa 1989
Sitana ponticeriana	Apr-Sept	Tirupati	Subba Rao & Rajabai 1972
	Apr- Sept	Dharwad*	Shanbhag & Radder (unpublished observations)
Mabuya carinata	Oct-Dec	Mysore	Sarkar & Shivanaadappa 1989
*Mabuya macularia**	June	Chennai	Daniels 2000
Varanus monitor	June-Aug	Jaipur	Jacob & Ramaswami 1976
Xenochrophis piscator	Jan-May	Calcutta	Saha et al. 1984
Lissemys punctata granosa	July-Aug	Varanasi	Singh 1974
Lissemys punctata punctata	July-Aug	Calcutta	Sarkar et al. 1996a

* *Seasonal ovarian cycle is not studied.*

Hemidactylus frenatus (Jones & Summers 1984), *H. brooki* (Shanbhag et al. 1998) and *C. versicolor* (Shanbhag & Prasad 1993a) of which the first three species are monoautochronic and the fourth one is polyautochronic. In *C. versicolor* ovaries the extrastromal follicles are organized into groups of similar sized follicles resulting in a size hierarchy amidst the groups. (Shanbhag & Prasad 1993a). Seasonal changes in size frequency distribution, the number of normal and atretic follicles belonging to different size groups (figure 13) revealed that the smallest follicles (Stage I: 0.5-0.75mm) are found throughout the year while recruitment of Stage II (0.76-1mm) and Stage III (1.0-2.0mm) previtellogenic follicles occurs from December to March. The vitellogenic (stage IV: <2.5 mm) follicles are recruited during late March and early April. At a given point of time, the ovaries contain only one group of vitellogenic follicles. After ovulation of a set of follicles and following midgestation, a next set of follicles is recruited for vitellogenesis (Shanbhag & Prasad 1993a, Radder et al. 2001). The mechanisms controlling recruitment of the second set of vitellogenic follicles are unknown at present. The previtellogenic atretic follicles are found throughout the year. Atresia is more prevalent in Stage III follicles suggesting their elimination in good measure before they enter vitellogenic phase. Atresia of vitellogenic follicles is rare. A pattern of recruitment and growth of follicles in the ovary of *C. versicolor* is depicted in figure 14.

In *H. brooki* each ovary has one GB. This lizard breeds from October to June at Dharwad (15°17'N & 75°3'E) and has at least two clutches in a breeding season (Shanbhag et al. 1998). The breeding is asynchronous among female individuals. Hence, the individuals in a population are in (1) non- breeding phase- ovary without yolky follicles and / or CL; (2) preparatory phase (vitellogenic - ovary with yolky follicles with no CL; (3) breeding phase- ovary with CL, no yolky follicles and oviductal eggs (gravid females); and (4) post-breeding phase- ovary with regressed CL and without oviductal eggs. The follicular dynamics during ovarian cycle of *H. brooki* is depicted in figure 15. There are 5 normal follicles in each ovary during preparatory phase while 4 in other phases. In each phase usually one early stage atretic follicle (AF) is found. Since the number of normal follicles is constant in each phase, loss due to follicular atresia is apparently overcome by recruitment of new follicle (s) from the GB. The pattern of selection and recruitment of follicles from

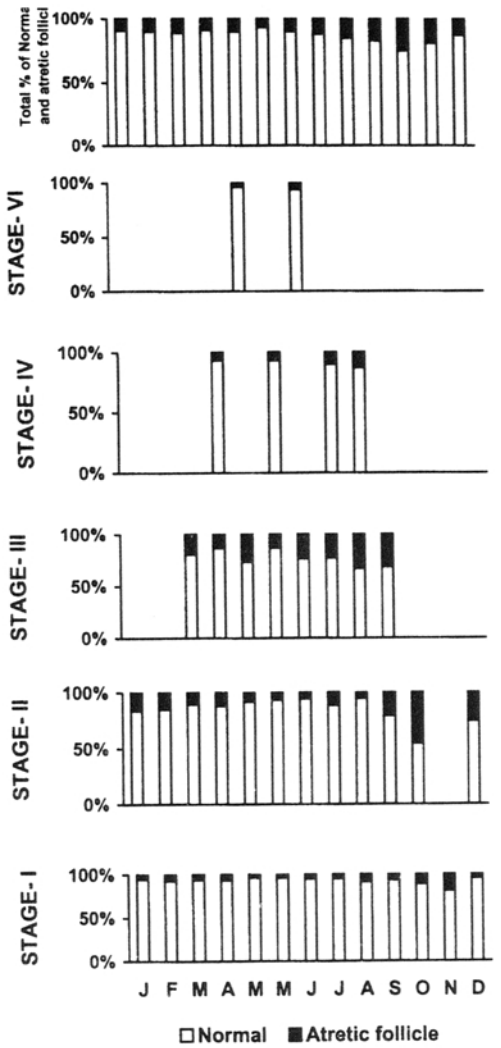

Figure 13 The percent of normal and atretic follicles of different stages, and total percent of normal and atretic follicles stages in the left ovary of *C. versicolor* during annual ovarian cycle. Stage I to III are previtellogenic and IV and V are vitellogenic follicles. Note that vitellogenic follicles when present are of only one stage. (from Shanbhag & Prasad 1993a)

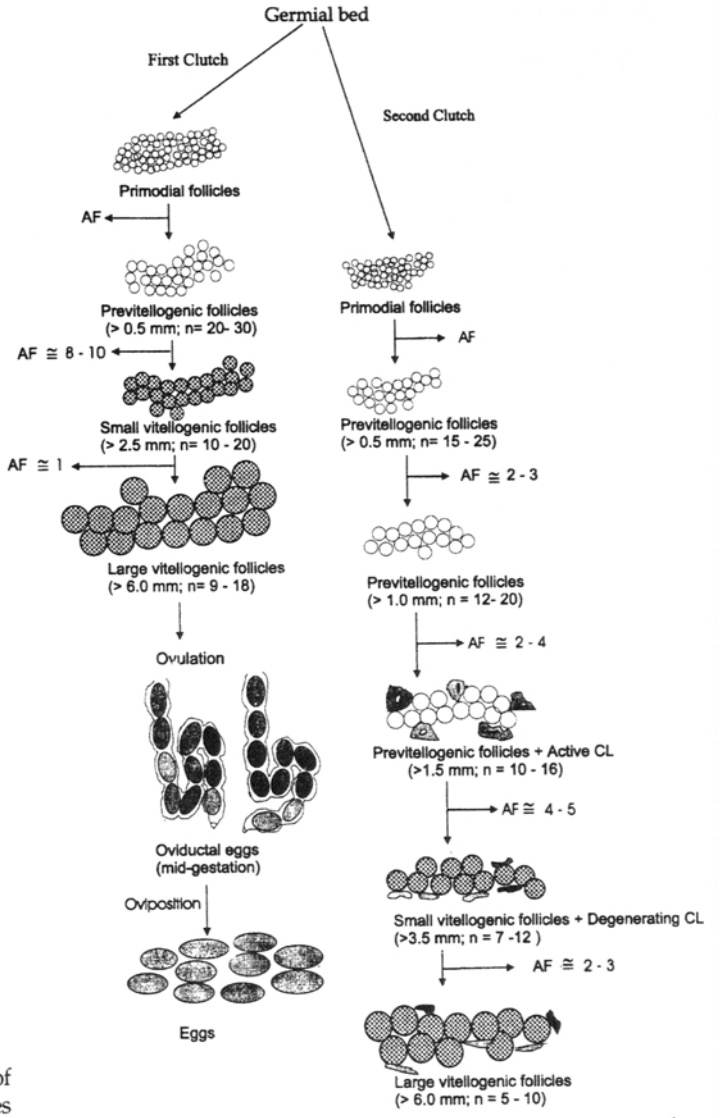

Figure 14 Correlative changes in the pattern of recruitment and growth of two consecutive clutches in *C. versicolor*. Clear circle- previtellogenic follicles, Dotted circle-vitellogenic follicles, triangular shaped- CL

GB seen in *L. lugubris* (Jones et al. 1978) and *H. frenatus* (Jones & Summers 1984) differs from *H. brooki*. In *H. brooki*, one follicle undergoes atresia in each phase of the ovarian cycle, while in *H. frenatus* only one follicle undergoes atresia during vitellogenic phase and in *L. lugubris* one or two follicles degenerate in a cycle. In *H. brooki*, 4 follicles are recruited in each ovarian cycle, but one reaches ovulatory size and undergoes ovulation. The other 3 degenerate. The next ovarian cycle is initiated with the recruitment of a new set of follicles in the breeding phase (Shanbhag et al. 1998).

Pattern of Changes in the Plasma Estradiol-17β (E_2) and P Levels during an Ovarian Cycle

The plasma sex steroids have been measured in several species of female reptiles and data obtained do not seem to follow a consistent trend even within the phylogenetic taxa (Duvall et al. 1982, Licht 1984). The information on pattern of plasma E_2 and *P* during ovarian cycle is available for soft shelled turtle, *L. p. punctata* (Sarkar et al. 1996a) and for the lizard, *C. versicolor* from India (Radder et al. 2001). In soft-shelled turtle which is single clutched, plasma E_2 levels rise with the growth of vitellogenic follicles, reaches its peak during breeding period

and falls following ovulation. In contrast, plasma P remains low throughout the ovarian cycle except for a postovulatory peak before oviposition (Sarkar et al. 1996a). Thus, a temporal association between ovarian activity, plasma E_2 and P is found in the turtle. In *C. versicolor*, during post-breeding and early recrudescence phases, both plasma E_2 and P are minimal (figure 16). In late recrudescent phase, a rise in plasma E_2 is associated with the growth of ovarian follicles. The levels of E_2 reach their peak values in individuals with large preovulatory vitellogenic follicles. The plasma P remains low in these individuals. Following ovulation, E_2 levels drop significantly while a steady increase in plasma P is observed. In individuals in early gestation stage, when eggshells are formed, plasma E_2 is low but P levels are high. During a breeding phase in multiclutched *C. versicolor* two reproductive events (vitellogenesis of a set of follicles and gestation) overlap in each lizard. In individuals in mid-gestation stage, with the onset recrudescence of second set of vitellogenic follicles, plasma E_2 levels once again rise and plasma P begins to drop. Thus, there is an inverse correlation between E_2 and P levels in such individuals during breeding phase. However, the plasma P remains low in both vitellogenic and non-vitellogenic individuals in late gestation stage. Since P levels are low following mid-gestation period, it is suggested that in *C. versicolor* P is not needed for oviductal egg retention during later part of gestation (Radder et al. 2001). Further, a drop in P levels around mid-gestation may actually facilitate the recruitment of a new set of vitellogenic follicles. Parallel studies in other Indian reptiles especially in monoautochronic species, ophidians, chelonians and crocodiles are needed to elucidate the pattern of plasma sex steroid levels during the ovarian cycle.

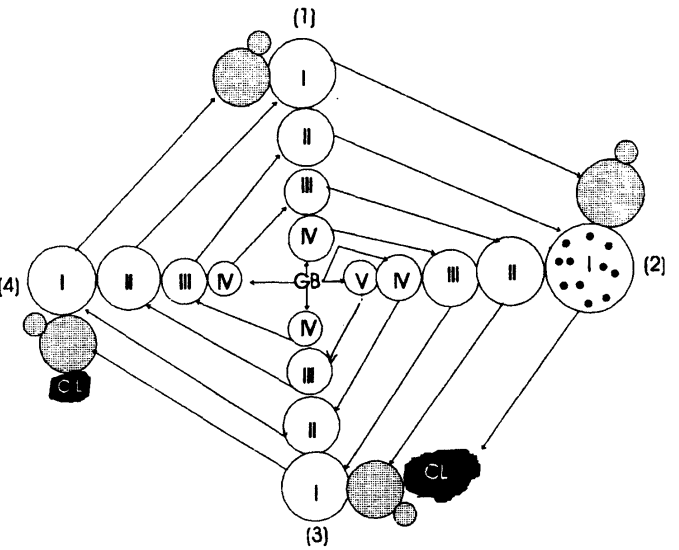

Figure 15 Schematic diagram showing the pattern of follicular recruitment and growth, atresia, and ovulation during the ovarian cycle in *H. brooki*. (1) Nonreproductive, (2) Preparatory, (3) Breeding and (4) Postbreeding phases. GB- germinal bed, Clear circle- developing follicles, Dotted circle- vitellogenic follicle; Shaded circle- atretic follicles, CL- corpus luteum (from Shanbhag et al. 1998)

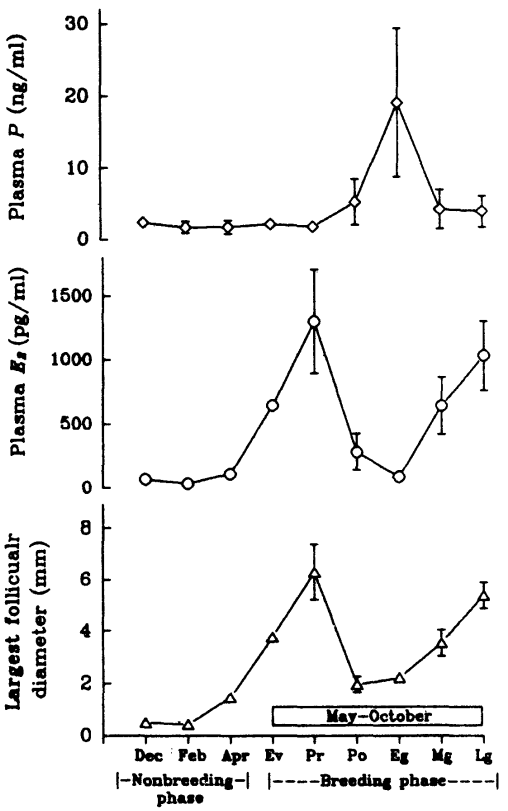

Figure 16 Changes in plasma E_2, P levels and largest follicular diameter (mean ± SE) during non-breeding and breeding phases in *C. versicolor*; Ev = early vitellogenic, Pr = preovulatory, Po = postovulatory, Eg = early gestation, Mg = midgestation and late gestation or pre-oviposition stages during breeding phase (from Radder et al. 2001)

Oviducts

Structure and Cycle

The paired oviducts are suspended by mesenteries from the dorsal body wall, and lie lateral to the ovaries. The reptilian oviduct exhibit three distinct regions (1) anteriormost part- infundibulum, (2) middle part- uterus, and (3) the posterior part- the vagina. The infundibulum opens anteriorly into

peritoneal cavity through ostium with fimbriated margin. The ovulated eggs are collected through ostium. The fertilization of eggs and their investment with albumen and eggshell take place within the infundibulum. Eggs are retained in uterine region until oviposition. It is muscular and glandular. This region accounts for almost two third of the oviduct. Uterine glands are greatly hypertrophied and secretory during breeding season. The vagina is short and relatively thinner. The two vaginae of either side open independently into the cloaca.

The oviducts show seasonal changes in their mass and morphology that is correlated to the ovarian cycle in *H. flaviviridis, C. versicolor* (figure 17), *P. dorsalis* and *M. carinata* (Haider 1985b, Sarkar & Shivanandappa 1989) and *L. p. punctata* (Sarkar et al. 1995). Oviducts hypertrophy during breeding phase and regress after breeding. In *H. flaviviridis* FSH induced oviductal hypertrophy during prebreeding phase (Haider 1985b). In *M. carinata* a decrease in weight and secretary activities of the oviducts following ovariectomy during breeding phase could be restored by the administration of E_2 and P. Similarly, oviductal hypertrophy was also induced by administration of E_2 and P during non-breeding period in the skink (Sarkar & Shivanandappa 1989). These studies indicate the role of E_2 and P in oviductal growth and function.

Oviductal Sperm Storage

Several species of reptiles are known to store sperm in the oviduct for a variable length of time (Angilini & Ghiara 1984). In *C. versicolor* and *P. dorsalis* (Sarkar & Shivanandappa1989, Shivanandappa et al. 1999) mucosal folds at uterovaginal junction become deeper to form sperm receptacles (figure 18) that are irregular in shape. The receptacles are lined by ciliated columnar epithelium interspersed with mucus secreting goblet cells (Shanthakumari et al. 1990, Srinivas et al. 1995). The sperm and secretary material are found as masses in the lumen of sperm storage pockets. Secretory granules in sperm pockets are PAS positive and also stain for proteins. Since these granules resemble the secretary granules isolated from epididymis and vas deferens, these are believed to be derived from males during copulation (Shivanandappa et al. 1999). Both *C. versicolor* and *P. dorasalis* store sperm as long as six months (Shanthakumari et al. 1990, 1992, Srinivas et al. 1995). These lizards exhibit extended breeding season producing more than one clutch. These lizards may just mate once during breeding period, thus reducing high risk of predation in the process of finding a mate, second time. Thus, the sperm storage mechanism seems to be an adaptation to ensure fertilization of eggs of subsequent clutches. However, survival of sperm for a long period in the oviduct is an intriguing phenomenon. Fertilizing ability of stored sperm is demonstrated in *C. versicolor* (Shanbhag & Prasad 1993b). The vitellogenic females collected from the wild and maintained in the laboratory in isolation from males were induced to ovulate by injecting PMSG. The eggs were not only fertilized by the stored sperm, but also successfully continued

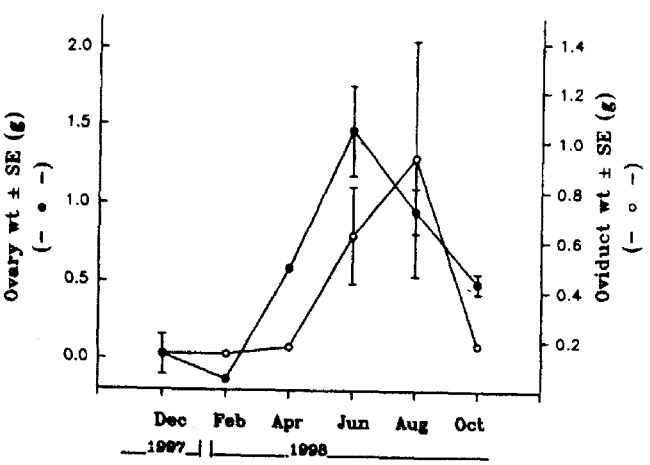

Figure 17 Shows correlative changes in the ovarian and oviduct weights in annual reproductive cycle in *C. versicolor.*

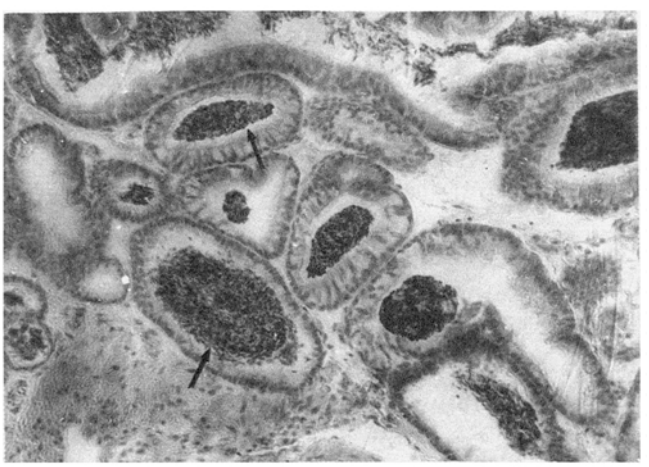

Figure 18 Section of utero-vaginal region of oviduct of *C. versicolor* showing sperm

embryonic development thereby confirming the utilization of stored sperm from sperm receptacles of the oviduct (Shanbhag & Prasad 1993b).

Endocrine Regulation of Ovarian Cycle

The work on endocrine regulation of ovarian functions is mostly confined to reptiles of temperate zones. Corresponding studies on reptiles inhabiting Indian subcontinent are meager. Like in other vertebrates, hypophysectomy causes degeneration of growing follicles in squamates while administration of pituitary extract or mammalian GtH reverse the effect of hypophysectomy (Duvall et al. 1982, Whittier & Crews 1987). In the quiescent phase, mammalian FSH and not LH induced ovarian growth in *H. flaviviridis* (Rai & Haider 1989). The treatment with FSH for a week caused increase in the number of oocytes, primordial and previtellogenic follicles; continuation of treatment for 21 days induced even vitellogenesis. Further, in FSH treated lizards, no atretic follicles were found. Similarly, in *M. carinata* administration of bovine FSH in the post-breeding quiescent phase caused an increase in the number of oogonia, oocytes and induced vitellogenesis, and elevated plasma E_2 levels (Nijagal & Yajurvedi 1999a). In *C. versicolor* PMSG induced ovulation during breeding phase (Shanbhag & Prasad 1993b). These studies suggest that in lizards mammalian FSH-like hormone performs the functions attributed to FSH and LH in higher vertebrates.

Circumstantial evidences derived from studies on annual ovarian cycle and experimental manipulation of the ovary in *C. versicolor* suggest that pituitary GtH is needed for oogonial proliferation and oogenesis. Increase in the number of oogonia and primary oocytes in the germinal bed coincides with preparatory and early breeding phases in the lizard (Shanbhag & Prasad 1993a). Unilateral ovariectomy (ULO) during post-breeding phase does not induce compensatory hypertrophy of contralateral ovary (COH), while during preparatory phase it induces COH due to increase in the size of follicles (Shanbhag & Prasad 1993c). Variation in the response of the ovary to ULO in the two different phases of the reproductive cycle is attributed to expected differences in the levels of GtH prevailing in the particular season i.e. low levels during post-breeding phase and high during prebreeding and breeding phases. Unfortunately, the work on correlative changes in plasma GtH levels and ovarian activity is lacking among Indian reptiles.

Several types of stress are known to induce increased corticosteroid secretion which seems to adversely affect gonadal functions, possibly through inhibition of GtH or by direct effect on gonads in reptiles (Greenberg &Wingfield 1987). Correlative changes between the ovarian and adrenocortical cell activities during the annual reproductive cycle are reported in *C. versicolor* (Vankudre & Shanbhag 1989). Interestingly, adrenocortical cells regress following ovariectomy during recrudescence phase which could be reversed by E_2 administration (Vankudre & Shanbhag 1992). In *M. carinata,* on the other hand, corticosterone caused a decrease in the number of oogonia in GB and primordial follicles in a dose dependent manner (Nijagal & Yajurvedi 1999b). High dose (40μg/lizard) of corticosterone arrested bovine FSH induced ovarian growth and vitellogenesis (Nijagal & Yajurvedi 1999a). The above studies indicate that corticosteroids affect ovarian follicular development and physiology but the nature of interrelationship between adrenal and ovary needs further investigation.

Strategies in Clutch Patterns and Egg size

Egg laying pattern seems to have a long phylogenetic history in each taxa. Most crocodiles and turtles lay large number of eggs compared to squamates. Broadly, the squamates fall into two categories in terms of their egg-laying pattern. The species of category I lay fixed number of eggs in a clutch (i.e. with determinate clutch or fixed clutch). For example, most geckos have a clutch size of two eggs. In contrast, squamates of category II lay variable number of eggs in a clutch (variable clutch). In individuals with variate clutch pattern (*C. versicolor, P. dorsalis*), the clutch size and frequency (especially in the tropics) may vary with proximate climatic factors, food availability, fat reserves of the body and maternal body size (Ballinger 1978, Schwarzkopf 1994). Several studies especially on chelonians and squamates describe the various mechanisms of achieving optimal reproductive

fitness through manipulation of clutch and/ or egg sizes (Ballinger 1978, Schwarzkopf 1994, Sinervo 1994). There are species in which the egg size is optimized in a population irrespctive of female's body size (Congdon & Gibbons 1987). Pelvic constraint may be one of the factors leading to the optimization of egg size. However, in some species of turtles and lizards (Congdon & Gibbons 1987, Michaud & Echternacht 1995) the egg size is not optimized and it varies with the body size of the female. It has been shown in these species that an increase in the body size is accompanied by a corresponding increase in the pelvic aperture.

Recently, Shanbhag et al. (2000c) investigated in detail the strategy of fecundity manipulation during breeding season by *C. versicolor* as a representative of Indian reptiles. They showed that the clutch size and clutch mass exhibit positive correlation with body size (figure 19) indicating that reproductive investment is influenced by females' body size. Further, they showed the clutch size and egg size are inversely related (figure 20). Thus, a trade-off between clutch size and egg size (volume) is obvious in *C. versicolor* and that there is no optimization of egg size. More interestingly, the above study also showed that the total energy allocated for reproduction (clutch mass) remains the same in early, mid and late breeding individuals (figure 21) even though the fecundity (clutch size) declines in late breeders. Apparently, the energy allocated for reproduction in each bout of egg production in *C. versicolor* is optimized by natural selection provided other conditions such as food availability are optimal and predator pressure is minimal. Further, in this lizard SVL is the prime factor determining the clutch mass (Shanbhag et al. 2000c).

The above study also reported that width of the pelvic aperture remains virtually constant over entire range of body sizes after attainment of sexual maturity in *C. versicolor*. Yet, they produce eggs of

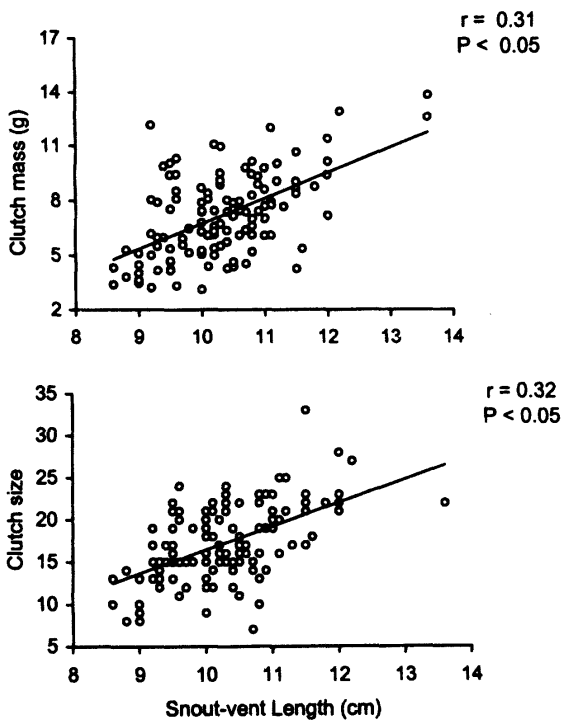

Figure 19 Shows the positive relationship between body size and clutch size and mass in *C. versicolor*

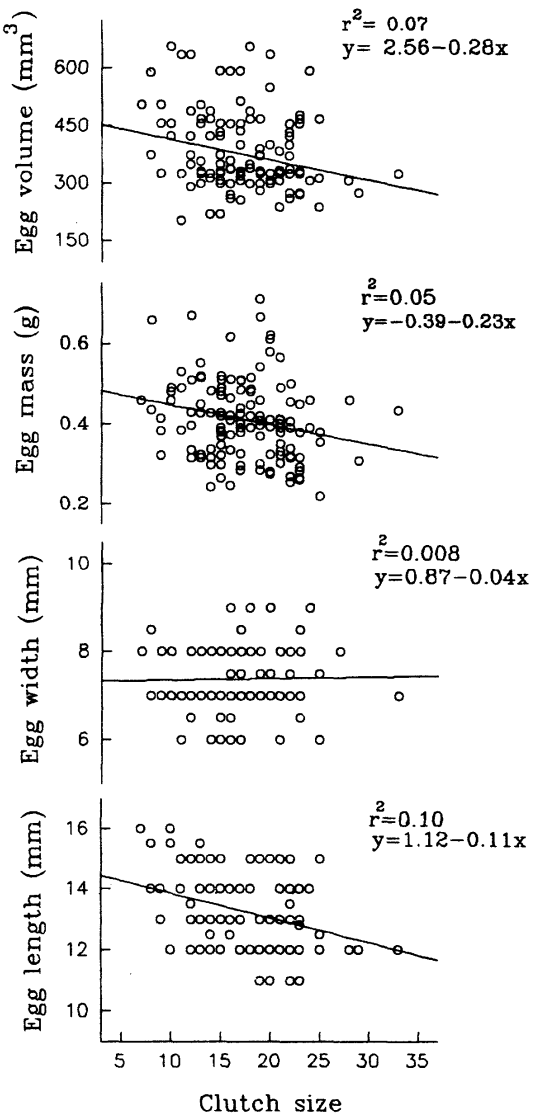

Figure 20 Shows the relationship between clutch size and egg length, width, mass and volume in *C. versicolor* (from Shanbhag et al. 2000c)

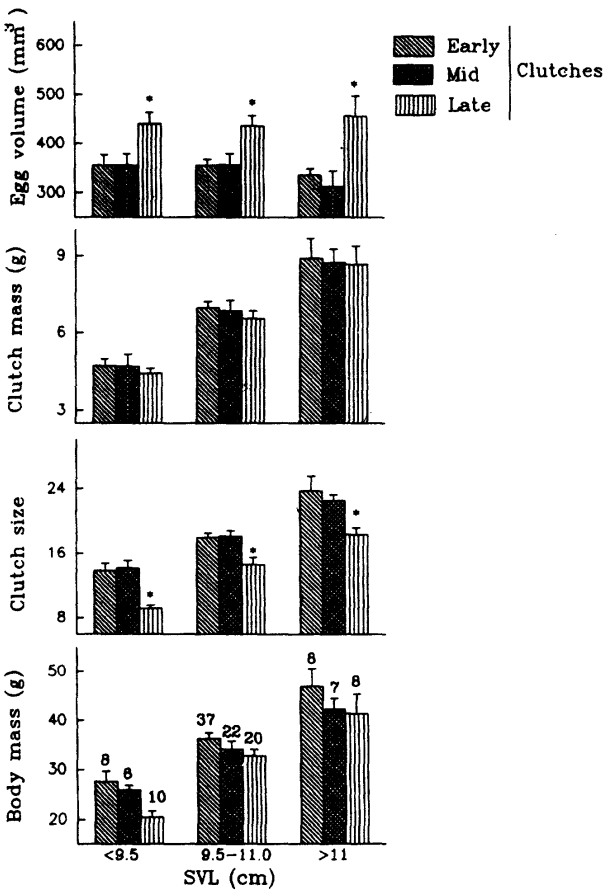

Figure 21 Changes in mean body mass, clutch size, clutch mass, and egg volume in lizards of different SVL groups with respect to early, mid and late clutches in *C. versicolor* (from Shanbhag et al. 2000c)

variable sizes by changing their length rather than the width over the entire range of SVL (table 3) to overcome pelvic constraint. Like in most oviparous vertebrates, in lizards, the mother deposits extra yolk in the egg, beyond what is absolutely necessary for the development of embryo until hatching stage. Interestingly, though there is a lot of variation in egg size with respect to breeding timing in *C. versicolor*, there is not much variation in the hatchling size (SVL). But the larger eggs (late breeding season) produce heavier hatchlings and these possess more internalized yolk than those born early in the breeding season (May-June) (Radder et al. 2002b). Producing heavier hatchlings with more internalized yolk at the end of the breeding season may enhance offspring's fitness since late born hatchlings have to compete for food and other resources with older hatchlings of early clutches.

Table 3 *Length and width of eggs in Calotes versicolor in early, mid and late clutches with respect to SVL (n = 126).*

SVL (cm)		Clutches		
		Early (May-June)	Mid (Jul.-Aug.)	Late (Sept.-Oct.)
< 9.5	L	13.18 ± 0.13	12.66 ± 0.21	14.75 ± 0.53*
	W	7.18 ± 0.18	7.16 ± 0.30	7.60 ± 0.24
		(8)	(6)	(10)
9.5 - 11.0	L	12.98 ± 0.17	12.72 ± 0.18	13.75 ± 0.23*
	W	7.39 ± 0.10	7.22 ± 0.17	7.70 ± 0.16
		(37)	(22)	(20)
> 11.0	L	12.81 ± 0.33	12.93 ± 0.07	14.00 ± 0.42*
	W	7.31 ± 0.13	7.29 ± 0.38	7.81 ± 0.20
		(8)	(7)	(8)

Role of Abdominal Fat Bodies and Reproduction

Abdominal fat bodies lying adjacent to ovaries or subcutaneous fat pads are reported in several species of squamates (Fox 1977, Licht 1984, Sarkar & Shivanandappa 1989). In lizards from semitemperate regions, the fat bodies are reported to serve as nutrient reserves during winter. An inverse relationship between the fat bodies and gonadal cycle has been reported in several species (figure 22) including Indian lizards, *C. versicolor, P. dorsalis* and *M. Carinata* (Sarkar & Shivanandappa 1989, Shanbhag & Prasad 1992, Sharma & Shanbhag 1992) In *H. brooki* the fat bodies are absent (Shanbhag & Saidapur 1994). Shanbhag and Prasad (1992) reported an arrest of recruitment of vitellogenic follicles following fatectomy in *C. versicolor* during preparatory phase. Conversely, ovariectomy (OvX) during the same phase induced an increase in the fat body mass. However, a significant decrease in the fat body mass is noted by E_2 treatment to the OvX lizards. Also, the fat bodies become reduced to traces with the advancement of breeding season in *C. versicolor*. These findings indicate that the lipids stored in the fat bodies are utilized for the development of first clutch of eggs. Food is generally abundant during late breeding season and it supports the growth of subsequent clutches of vitellogenic follicles (Shanbhag & Prasad 1992). Further studies are needed to elucidate the functional relationship between fat bodies and ovarian development, if any, in reptiles.

In male *C. versicolor*, FBX does not affect spermatogenesis (Sharma & Shanbhag 1992). However, a significant reduction in fat body mass

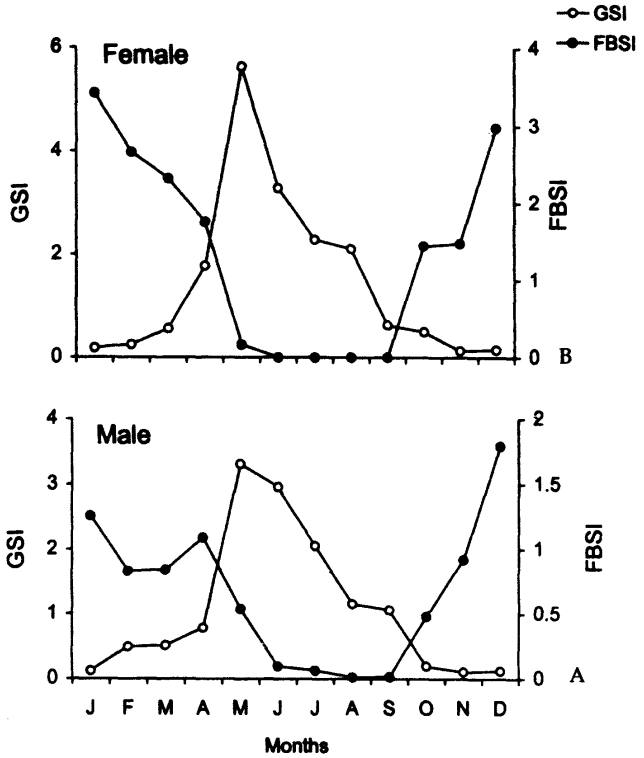

Figure 22 Correlative changes in GSI and FBSI in male (A) and Female (B) during annual reproductive cycle in *C. versicolor* (adopted from Sharma & Shanbhag 1992 and Shanbhag & Prasad 1992)

occurs in wild lizards during breeding period. Therefore, mobilization of their lipids from fat bodies during breeding season takes place probably to meet energy demands for pre-reproductive events such as mate selection, courtship, territory guarding, male-male combat etc. The fat bodies in lizards may play a supportive role in reproduction especially during periods of unpredictable or fluctuating resource availability. Although a seasonal breeder, *H. brooki* does not store lipids in the form of fat bodies or fat pads. Presumably this reflects the presence of a relatively constant food supply to house geckos.

Environmental Control of Gonadal Cycles

Role of Physical Environmental Factors

Our knowledge of the environmental factors affecting gonadal activity is mainly derived from studies on temperate lizards (Licht 1984, Duvall et al. 1982, Whittier & Crews 1987). Photoperiod was traditionally considered important variable affecting reproduction in lizards. But in recent years, temperature is believed to be the most important factor (an initial predictor) in controlling gonadal recrudescence. The absolute levels of the body and environmental temperatures of a species determine whether it will have an acceleratory or inhibitory influence on gonads. The photoperiod appears to be a causal variable associated with cyclic activity of the gonads (Licht 1984, Wingfield & Kenagy 1991). Other stimuli such as rainfall, relative humidity, food supply and conspecific members of opposite sex also play an important role in fine- tuning of gonadal maturation, mating and oviposition.

But for one study reporting photothermal effect on ovarian cycle in *L. p. punctata* (Sarkar et al. 1996b), studies on the environmental control of gonadal cycles are virtually lacking for Indian reptiles. In *L. p. punctata*, high temperature is instrumental in triggering ovarian growth during preparatory phase but it does not sustain its stimulatory effect and, rather causes degeneration of the ovary. The photoperiod, *per se*, does not seem to have any role in ovarian cyclic activity (Sarkar et al. 1996b). Our information on the role of environmental factors in regulation of gonadal cycles in lizards is mainly derived from phenological observations. Studies on the annual gonadal cycles on *C. versicolor* and *P. dorsalis* show an association between rise in temperature (from February-March) and gonadal recrudescence. Oviposition is timed with monsoon rains. Both these species exhibit extended breeding season and therefore produce more than one clutch during a breeding season. The eggshell of these species is chitinous and, moisture is essential to prevent desiccation of developing embryos. In contrast, the house lizards, both *H. flaviviridis* and *H. brooki* breed during the dry months of the year. Their eggshells are calcareous and do not face the problem of desiccation. However, *Mabuya maculatus* breeds in July, monsoon months in Chennai produces eggs with calcareous shells (Daniels 2000). These findings suggest that the eggshell composition in the lizards has a long phylogenetic history. The breeding timing of two species geckkonids studied from India also varies. In southern India *H. brooki* exhibits an extended breeding season and gravid specimens are encountered from October to June. On the other hand, in northern India where winter

temperature plummets greatly *H. flaviviridis* exhibits short ovarian cycle in summer, prior to monsoon. Apparently, temperature plays an important role in ovarian recrudescence and growth in the lizards.

Role of Socio-Sexual Factors

Social cues from conspecies serve as important regulators of reptilian reproductive cycles. It has been shown explicitly that the presence of male and courtship behavior is needed for normal ovarian recrudescence and fecundity in *Anolis carolinensis* (Crews 1990, Summers et al. 1995). The effect of socio-sexual factors on gonadal recrudescence and plasma steroid hormone levels has been recently studied in *C. versicolor* (Shanbhag et al. 2002). The study showed that isolation of lizards from the opposite sex adversely affects gonadal recrudescence. Presence of a female, irrespective of its gonadal state can initiate spermatogenesis but maintenance of prolonged spermatogenetic activity needs the physical association (tactile stimuli) of female in breeding condition. Similarly, in females, contact with the males with spermatogenetically active testes with high T titers is needed for optimum growth of vitellogenic follicles. The study thus illustrates the relative importance of chemical/ olfactory, visual and tactile cues (arising from the members of the opposite sex) in optimum gonadal growth in *C. versicolor.* The precise role of socio-sexual factors in reproduction of other reptiles needs to be investigated in detail.

Post-breeding Refractory Period

In seasonally reproducing lizards and snakes from temperate zones, it is shown that post breeding gonadal quiescence may result from refractory condition whereby the animal is rendered wholly or partially insensitive to stimulation by exogenous factors like temperature, photoperiod and humidity (Licht 1984). Administration of mammalian GtH to *C. versicolor* (Gaitonde & Gouder 1985) and *M. carinata* (Chandramohan & Yajurvedi 1995) during post-breeding phase induced testicular recrudescence. Therefore, post-breeding gonadal regression need not be related to the existence of a gonadal refractory period in these species, rather, this phase of cycle may simply result from the absence of appropriate stimuli from the environment. The recent study on *C. versicolor* showed that during postbreeding quiescent phase (Dec-Jan) the administration of mammalian GnRH at ambient temperature and at 30^0 C (temperature prevailing during recrudescent phase) induced testicular recrudescence and rise in plasma T (figure 23), while exposure to mere high temperature failed to induce testicular recrudescence (Shanbhag et al. 2000b). Since mammalian GnRH as well as GtH induced testicular recrudescence during post-breeding season in *C. versicolor,* it appears that the higher brain centers that regulate GnRH secretion from hypothalamus or hypothalamus become thermorefractory following breeding leading to low GtH output from hypophysis and testicular quiescence.

Captive Breeding and Management of Reptiles

There are around 456 species of reptiles in India of which 197 are endemic. Of these 214 are regarded as "threatened". Though many Zoos in India claim to be breeding a number of species of snakes, there is no information on the captive breeding and management of the "threatened" endemic species except the crocodiles. Rehabilitation is one of the

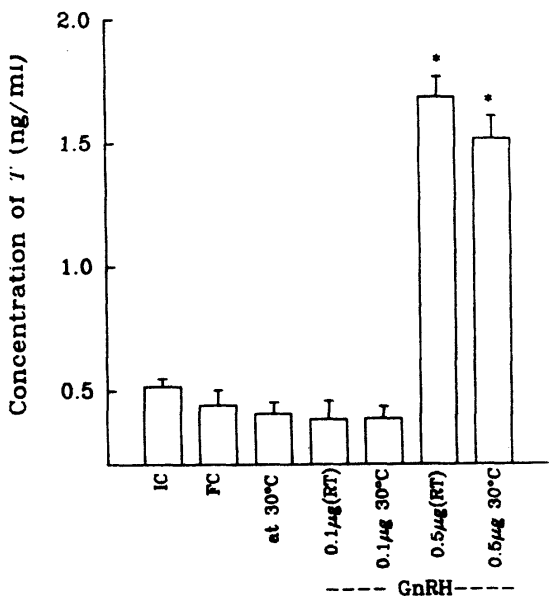

Figure 23 Plasma levels of T ± SE in different groups in *C. versicolor.* * indicates significant difference from rest of the groups. IC: Initial control, FC: Final control, RT: Room temperature (From Shanbhag et al. 2000b).

goals of captive breeding. The three species of crocodiles, mugger crocodile, *Crocodylus palustris*, salt-water crocodile, *C. porosus* and gharial, *Gavialis gangeticus* have been successfully bred in captivity and also released into the wild. Due credit goes to Madras Croc Bank for bringing these crocodiles and gharial back from the edge of extinction and restoring them to former abundance. This type of captive breeding and conservation programs are not taken up for other threatened species of reptiles. Therefore, there is a need to take up conservation and breeding for endemic species that are threatened. However, for a successful conservation and breeding program, there is a need to obtain the basic information from field studies about their habits and habitat, rearing of eggs/hatchlings. Such information will be of great help in captive breeding programs.

Reptiles' being shy and cryptic not much is known about their physiology, reproduction, sex differentiation and behavior. Reproductive biologists, ecologist and behavioral biologist are interested in understanding various aspects of reptilian biology that is very tough and tedious to study in natural population using mark- recapture methods. Captive breeding and management of reptiles of lower risk-least concerned (LR-LC) species will help us understand many aspects of their biology without disturbing the fauna from nature. Successful captive breeding and rearing also helps in following their lineage through successive generation. Keeping this view in mind, B. A. Shanbhag and her coworkers have initiated captive breeding of the common garden lizard, *C. versicolor* (which fall under LR-LC as per Red data book) since last four years. The lizards are maintained successfully in captivity. They lay fertilized eggs in terraria. The eggs have been successfully incubated and hatched in the laboratory. Hatching success is more than 90%. The hatchlings are then released in the botanical garden of the University. A few were also reared in the laboratory. of which one male and female each (10 moth old) are being reared until now (June 2002). The survival of neonates is important in all conservation-oriented programs, regardless of whether hatchlings are to be maintained in captivity or to be released into wild. A success in rearing the offspring till maturity and reproduction generates a great potential to progress in the field of reptilian behavior, reproduction etc. Also standardization of the protocols for captive breeding and management of select lizards in captivity will help us in conservation and management of desired species.

Concluding Remarks

We are beginning to understand the reproductive strategies of Indian lizards but more studies are needed on other groups of reptilian species. At present very little is known about the age at first reproduction, number of clutches in a breeding season, clutch size, absolute egg size relative to maternal body size and mass, age specific fecundity, survivorship of hatchlings to sexual maturity, reproductive life expectancy of adults (phenomenon of senility) and so on. These areas are neglected partly because their significance is not generally appreciated and partly because these are most difficult to study. Studies on reproductive cycle of different species living in a habitat or closely related species living in different habitats are essential to generate information about the reproductive strategies and their control (endocrine as well as environmental) mechanisms. Comparative data will provide an opportunity to study the mechanisms governing the evolution of diversity in reproductive patterns, strategies associated with the management of clutch size, clutch frequency, egg size, sperm storage, prolonged oviductal egg retention and so on. Such studies would provide clues not only to our understanding of reproductive processes but also the diverse mechanisms governing reproduction in reptiles, and finally the evolution of viviparity. The role of food and importance of con-specifics interaction in reproduction among reptiles is another aspect that has received very little attention. Such studies will be of great value in planning captive breeding and management programs for threatened or rare species.

Studies on gonadal sex differentiation and its control mechanisms are scanty among reptiles from India. The exact mechanism of TSD in the turtle *L. olivacea* and crocodile, *C. porosus* is not known. It would be interesting to know whether various

mechanisms proposed governing TSD among reptilian species from temperate region also hold good for those from Indian subcontinent. The study of life history of any organism is complete only when we fully understand all these aspects. Hopefully, in the near future we will gain more information on the various aspects of reproductive biology of Indian reptiles.

Acknowledgement

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