PREFACE
The endocrine role of the gonads and the adrenals of mammals has been a subject of considerable investigations and only in recent years, attention has been diverted towards the study of endocrine functions of the gonads and adrenals of the nonmammalian vertebrates, and the reptiles, surprisingly, are the least studied, though they are of considerable phylogenetic interest.

The reptiles are the pioneers amongst the land vertebrates that have developed internal fertilisation and amniotic egg. Being the most archaic amniotes, they have adapted themselves to the land life by developing diversity in their reproductive patterns. The male accessory organs and ducts, because of the introduction of internal fertilisation, have assumed an important role and thus have attained complexity. With the introduction of internal fertilisation, there is also an economy in the production of eggs which now need to be protected from the hazards of the Nature and the predators. Hence retention of eggs or embryos in the female ducts is a common feature amongst the reptiles. Considerable variation exists in their reproductive cycles. The reptiles of colder regions are the seasonal breeders with a breeding phase of short duration while those of the warmer regions have an extended breeding
phase that ensues in spring and lasts till the end of the summer season. Thus the reproduction in reptiles is timed with the environmental conditions. Evidently, the variations in their reproductive patterns are regulated by the endocrine mechanism that involves the action of hormones.

In vertebrates, in general, the gonads and adrenals or interrenals (and also placentas of mammals) are the sites of biosynthesis of steroid hormones. The Leydig cells and their homologues, the lobule boundary cells of the testis have been found to be the principal site of steroidogenesis. Only in recent years, the Sertoli cells have been claimed to possess the steroidogenic potential. The steroidogenic role of the ovary in the nonmammalian vertebrates has not been understood as much as its counterpart, the testis. The investigations are very few and the observations made thereon are rather divergent so that hardly any generalisation is possible. Generally, granulosa cells of the ovarian follicles are considered to be steroidogenic. There are also reports claiming that theca interna cells are steroidogenic. Even the oocyte of amphibians and birds have been found to show weak steroidogenic activity. There is not much information on the steroidogenic cells in the reptilian ovary.
Follicular atresia is a degenerative process of the ovarian follicles, common to all the vertebrate series. A number of follicles develop in the lower vertebrates showing external fertilization, obviously, to compensate the high mortality of the defenceless eggs and developing forms, and to increase the chances of their survival. The higher vertebrates, with the introduction of internal fertilization, retention of oviducal eggs and viviparity, produce a small number of eggs or embryos. Thus the majority of the ovarian follicles undergo follicular atresia in amniotes.

The atretic follicles have been claimed to possess transient steroidogenic activity. The theca interna cells, after the follicular atresia is over, are reported to contribute to the interstitial gland cells of the ovarian stroma in some vertebrates. There are also evidences to indicate that the ovarian stromal cells have steroidogenic potential. Some problems connected with follicular atresia in the vertebrates have remained unresolved. The endocrine regulation leading to follicular atresia in vertebrates is not clearly understood. Its role as an internal secretor of steroid hormones has not been conclusively proved. Even, the histological studies on follicular atresia do not cover all the representatives of the vertebrate series.
Follicular atresia in birds and fishes occurs in many ways and various types of atretic follicles have been recognised in birds. Only two types of follicular atresia — yolky and bursting atresia — in the reptilian ovary have so far, been described. Contribution of theca interna cells of the atretic follicles to the interstitial cells of the ovarian stroma is known only from a few studies which are not adequate to draw any generalised conclusion for the vertebrate group as a whole.

The ovarian follicles after the discharge of ova, continue as postovulatory follicles or corpora lutea for varying length of time. They are short-lived in lower vertebrates and are seldom steroidogenic. In the higher vertebrates that show retention of eggs or embryos, the corpora lutea remain functional till the period of oviposition or gestation. Thus the luteal bodies seem to be involved in the maintenance of eggs or embryos. These studies are limited and they do not permit any generalised conclusion for the reptilian group. Divergent findings have been reported on the origin of luteinised cells of the corpora lutea in reptiles.

Normal ovarian follicles of the vertebrates are also known to possess steroidogenic elements. Generally,
the cells of the granulosa and theca interna have been claimed to be involved in the biosynthesis of steroid hormones. The results of these studies are not unanimous, probably for the reason that the ovarian elements undergo cyclic changes in their structure. Moreover, no systematic investigations covering the different phases of the annual breeding cycle, has been carried out on the reptilian ovary.

As compared to ovary, the testis of the vertebrates has remained virtually unchanged. Throughout the vertebrate series, it produces a large number of sperms. With the introduction of internal fertilization, the evolutionary changes are discerned mainly in the male accessory organs and ducts that play a role during mating. In lower vertebrates that show external fertilization, the milt is released in one lot during spawning. Hence the spermatogenetic stages develop synchronously in lower vertebrates. In higher vertebrates, waves of sperm production take place as a result of asynchronous spermatogenesis since repeated mating takes place during the breeding season. Probably, for this reason, primumitial spermatogenesis takes place in the lower vertebrates while the higher vertebrates exhibit postunptial type in which spermatogenetic activity is resumed soon after the breeding season is over.
The cyclic changes in the structure of the testis in reptiles is fairly well documented. But there is still a need for establishing the correlation between the cyclic changes in the testicular elements and fluctuations in the environmental conditions. Some studies have been carried out to show the influence of temperature, rainfall on the reproduction in reptiles. Further, there is a need to investigate the Leydig cell and Sertoli cell cycles in reptiles.

There are evidences to show that the interstitial Leydig cells and to a lesser extent Sertoli cells possess the potentiality to synthesise steroid hormones. The observation of steroidogenic potency of the Sertoli cells is only recent and electron microscopic studies support this observation. The histochemical demonstration of steroidogenic cells in the testis of reptiles is limited only to a small number of reptilian species. The occurrence of 17β-MSH and 11β-MSH enzymes in the testis reptiles, is yet to be verified since the identification of steroids and their in vitro conversions tend to indicate the presence of these enzymes in the testis of reptiles. Hence there is a need for further work in this direction in reptiles.

The nonmammalian adrenals or the interrenals are
being increasingly investigated only in recent years. The histology and the cyclic changes in the adrenals of reptiles are fairly known. The steroidogenic ability of the adrenocortical or interrenal cells, has been investigated only in a few reptiles. The occurrence of some of the important steroid converting enzymes, namely, 17β-HSDH and 11β-HSDH, known to occur in the adrenals of mammals, are yet to be demonstrated in the adrenocortical cells of reptiles.

The cyclic changes in the structure of the gonads and adrenals, obviously, reflect the changes in their functional activity. Hence the cyclic changes in the steroidogenic activity in these organs of the reptiles is yet to be investigated.

In view of the above facts, the present work was planned to identify the steroidogenic cellular sites in the gonads and the adrenals of some reptiles by means of histochemical demonstration of some steroid converting enzymes that play a role during steroid biosynthesis. This work is only a part of series of investigations some which have been completed and several papers have been published from our laboratory on the steroidogenic cells of the nonmammalian gonads and adrenals. The reptiles in
particular have been chosen in the present work because of several reasons, foremost amongst them being the fact that reptiles are the most archaic amniotes with considerable diversity in their reproductive patterns.

The results of our study, presented in this thesis envisage (1) the histology of the ovaries, testis and adrenals of some reptiles, (2) seasonal changes in the structure of the ovaries and the testis of *Calotes versicolor* as the representative reptile, (3) histochemical demonstration of steroidogenic cellular sites in the ovaries, testis and adrenals of some reptiles and (4) the seasonal changes in the activity of steroid converting enzymes in these organs in *C. versicolor*. For the sake of convenience, the work is presented in three parts in the thesis:

**PART I : THE OVARY (CHAPTER 1-3)**

Chapter 1 deals with the histology of the ovaries of *C. versicolor*, *Hemidactylus flaviviridis* and *Chamaeleon calcaratus*. The structure of the germ bed, normal follicles, postovulatory follicles, atretic follicles and the ovarian stroma has been described. Some types of follicular atresia, not reported before, have also been described.
Chapter 2 deals with the monthly studies on the ovarian changes in *C. versicolor*. An attempt is made to establish the correlation between the ovarian changes and the seasonal fluctuations in temperature, relative humidity, rainfall and day length.

In Chapter 3, our observations of the steroidogenic elements in the ovary of a representative reptile, *C. versicolor*, carried out for one annual cycle are given. An attempt is also made to study the seasonal changes in the activity of \( \Delta^5-3\beta-\text{HSDH}, 17\beta-\text{HSDH}, 11\beta-\text{HSDH}, \text{G-6-PDH}, \) NADH diaphorase, LDH and ICDH enzymes in the steroidogenic elements of the ovary of *C. versicolor*.

**PART II : THE TESTIS (CHAPTER 4-6)**

Chapter 4 includes the comparative account of the structure of the testes in *C. versicolor*, *H. flaviviridis*, Habuya carinata, *C. calcaratus*, *Python molurus*, *Naja naja* and *Crocodilus porosus*.

Chapter 5 deals with the seasonal changes in the testicular elements of *C. versicolor* as observed from the monthly histological studies. Monthly morphometric data,
namely, gonosomal index, testis diameter, seminiferous tubule diameter, height of the seminiferous epithelium, the cell and nuclear diameters of the Leydig cells and Sertoli cells in the testis of *C. versicolor* have been measured in order to understand the testicular changes. As in the study on the reptilian ovary, an attempt is also made to understand the correlation of the cyclic changes in the testis of *C. versicolor* with seasonal changes in temperature, relative humidity, rainfall and day length.

Chapter 6 pertains to our histochemical observations of \( \Delta^5-3\beta \)-HSDH, 17\( \beta \)-HSDH, 11\( \beta \)-HSDH, G-6-PDH, NADH diaphorase, LDH and ICDH enzymes in the testes of *C. versicolor*, *H. flaviviridis*, *H. carinata*, *P. molurus*, *N. naja*, and *C. porosus* and the monthly studies on the histo-chemical demonstration of these enzymes in the testis of *C. versicolor*. An attempt is also made to study the correlation between the changes in the activity of these enzymes in the testis of *C. versicolor* and the fluctuations in temperature, relative humidity, rainfall and day length.

**PART III : THE ADRENALS (CHAPTER 7)**

In Chapter 7, the observations on the structure of adrenal glands in *C. versicolor*, *H. flaviviridis*,
*M. carinate*, *C. calcaratus*, *P. molurus*, *N. naja*, and *C. porosus* are given. Further, histochemical demonstration of the steroid converting enzymes has also been carried out on the adrenals of these reptiles. The results of our observations on the cyclic changes in the activity of the steroid converting enzymes in the adrenals of *C. versicolor* are also given in this Chapter. The chromaffin cells which are considered to be the homologues of the mammalian adrenomedullary cells that secrete adrenalin and noradrenalin, have also been histochemically examined in the adrenals of these reptiles.

As this preface is intended only to serve as a general introduction for the thesis, no endeavour is made to quote the references of earlier studies on the subject. However, there is a detailed introduction and discussion for each Chapter of the thesis in which relevant reference listed at the end are cited.

The inferences drawn from the present histological and histochemical studies need further confirmation based on the studies such as in vivo and in vitro conversion of steroids in the gonads and adrenals, identification and quantitation of plasma steroids and electron microscopic observations. Due to limitations of laboratory facilities
such as non-availability of sophisticated equipment and fine chemicals, these studies could not be carried out in our laboratory. Efforts are being made to equip the laboratory with sophisticated instruments and we look forward to carry out further work in the field of comparative endocrinology and reproduction.