INTRODUCTION

Reproduction is an essential prerequisite for continuation of one's generation. It involves a multifunctional sequence of ecological and physiological activities. Changes in the biometeorological conditions signal the endocrine system, which in turn dictates the reproductive habits of the animals (Immelman, 1971; Lofts and Murton, 1973; Dawson, 1999). Seasonally breeding animals frequently detect and respond to cyclic environmental cues that accurately signal well in advance, the variations of seasonal periodicities which then favours reproductive success (Farner and King, 1983). Reproductive system gears up under the influence of hormones released inside the body in response to endocrine changes (Murton and Westwood, 1977; Etches, 1996). Hormonal mechanisms regulate and synchronize all aspects of reproduction, from the maturation of sperms and eggs to the expression of species-specific behavioral patterns that are employed to defend a territory, court a female and ensure the fertilization of eggs. Furthermore, hormones are known to coordinate the physiology and behaviour of an animal by regulating, integrating and controlling its bodily functions from its area of release. There is a gradual shift from the aggressive courtship behaviour to the nest-oriented and care-taking behaviour explaining the fact that hormones are required in the transition from one behaviour to the next (Nelson, 2000). As the significance of sex hormones in the initiation and maintenance of sexual behaviour by regulating, integrating and controlling bodily functions from its area of release in both males and females became apparent, in

Gonads are known to be the major sites for sex steroid biosynthesis. Testosterone or Androgens (male hormones) and Progesterone and Estradiol (essential female hormones) are released from the gonads into the blood stream for further eliciting reproductive functions (vander Molen and Rommerts, 1981; Hiller, 1985; Paster, 1991). Steroid metabolizing enzymes required for the formation of pregnenolone and for the further conversion of pregnenolone to androstenedione are mainly present in the theca and granulosa layer of ovaries and leydig cells of testes (Nalbandov, 1970). Significant histophysiological alterations in terms of gametogenesis and steroidogenesis are bound to be observed during the reproductive cycle of animals. The presence of enzyme activities in a particular tissue is one of the factors that determine conversion rate of reaction, which also depends on the presence of proper substrates, cofactors, induction, inhibition, stimulation of enzyme activities etc.

Of recent, organs other than the gonads and adrenals are being recognized as steroid synthesizing sites, *viz.*, liver, intestine, etc., (Farhting *et al.*, 1982; Lang *et al.*, 1986; Andersson, 1995; Andersson *et al.*, 1995; Ghraf *et al.*, 1975; Lateef *et al.*, 1997; Stupans *et al.*, 2000). In the wake of new found evidences of the role of liver and intestine in steroid biosynthesis in amphibians (Picariello *et al.*, 1982; Paolucci and Botte, 1988; Belverede *et al.*, 2001), investigations on the role of these tissues in aves were undertaken in the present work. Previous studies in our laboratory have shown the probable involvement of extra-gonadal tissues (liver, intestine and kidney) in steroid metabolism during annual reproductive cycle of Feral Blue Rock Pigeon, *Columba livia* (Kotak, 1979). The present study has been carried out for the purpose of furthering information on the involvement of extra-gonadal tissues in steroid metabolism at different phases of the reproductive cycle in two seasonally breeding species of birds. To understand the species-specific and sex-specific physiological differences, if found any, between seasonally reproducing birds, the two model bird species selected were: Bank Myna, *Acridotheres ginginianus*, and Brahminy Myna, *Sturnus pagodarum*. The reproductive cycle of these birds were divided into four phases *viz.*, pre-breeding, breeding, post-breeding and non-breeding phases, with inference from previous field studies (Thapaliyal, 1978; Khera and Khalsi, 1986; Parasara, 1989; Patel, 1991; Kumar and Kumar, 1993) as well as personal field observations in Baroda.

Convincing evidences of the role of liver, intestine and kidney as important sites for steroid metabolic pathways (Ambadkar and Kotak, 1978; Bhujle and Nadkarni, 1975, 1978; Farthing *et al.*, 1982; Antoun *et al.*, 1985; Nishinaka *et al.*, 1991; Elaroussi *et al.*, 1993; Martel *et al.*, 1994; Yammamuro *et al.*, 1994; Andersson, 1995; Andersson *et al.*, 1995; Lateef *et al.*, 1997; Harpaz and Uni, 1999) to support steroid metabolism in gonads are progressive signs in this field of research.

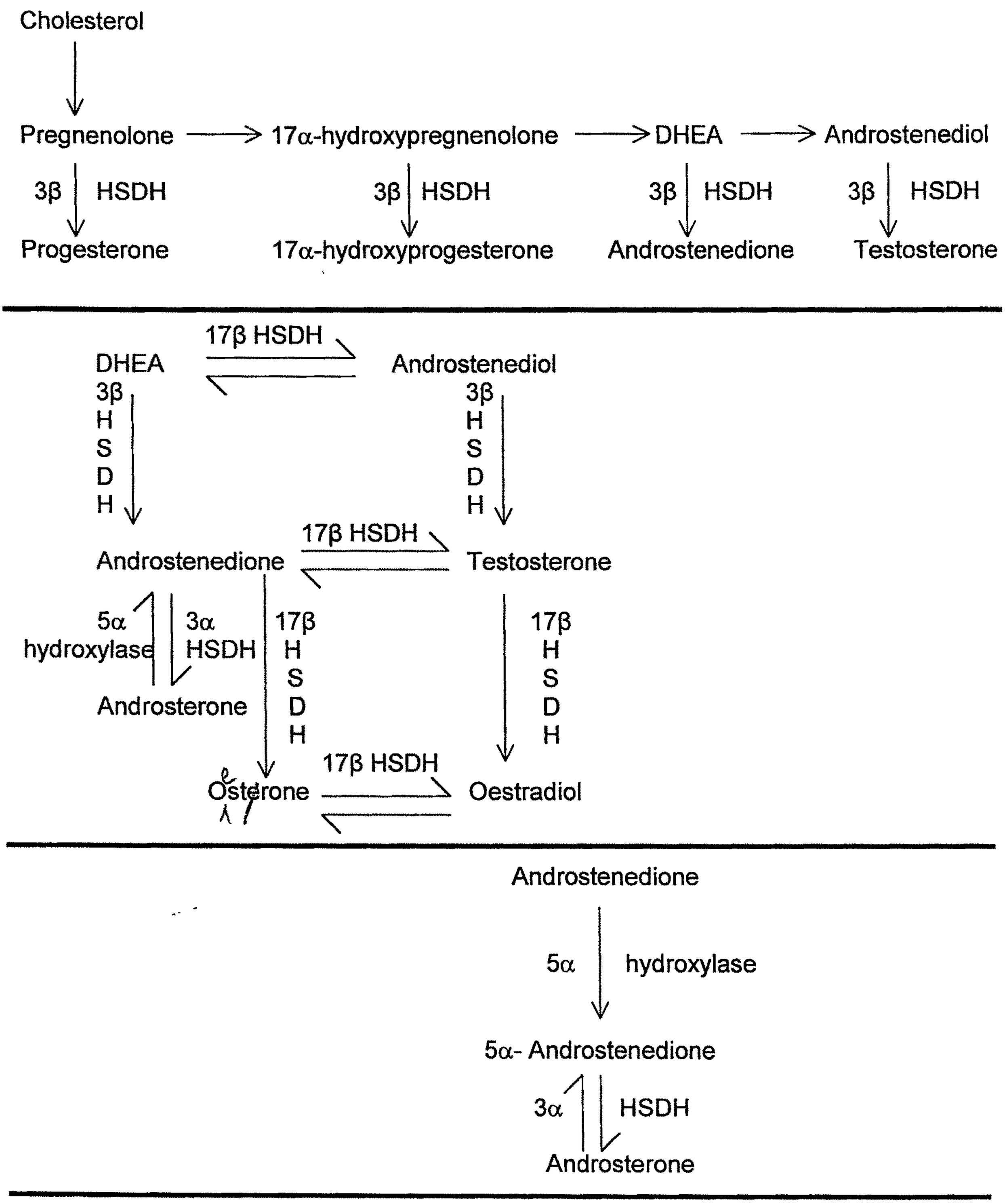
As a sequel to the above thoughts, studies on histoenzymological alterations of steroid dehydrogenases were initiated to establish the probability of the role of extra-gonadal tissues along with the gonads in these two species of Mynas. Changes in the intensities of the localization patterns of three steroid dehydrogenases: 3β-hydroxysteroid dehydrogenase (3β-HSDH), 17β-hydroxysteroid dehydrogenase (17β-HSDH) and 3α-hydroxysteroid dehydrogenase (3α-HSDH) which are known to be indicative of various levels of steroid hormone biosynthesis/metabolism (Baillie *et al.*, 1966) were investigated.

3β-HSDH is essential for the production of all classes of steroid hormones. It is now a well-established fact that it catalyzes the conversion of pregnenolone to progesterone in Δ^4-3-ketosteroid metabolic pathway and DHEA to androstenedione in the Δ^5-3βhydroxysteroid pathway (Baillie *et al.,* 1966)[Flow Chart 1]. Ultra structurally it has been demonstrated that the 3β-HSDH are localized on the tubular or lamellar cristae of mitochondria and smooth endoplasmic reticulum (Deray, 1976; Armstrong, 1979; Nagai, 1985) of ovulating follicles. Theca interna of developing, atretic and post-ovulatory follicles are the potential sites for 3β-HSDH localization in the ovaries of wild birds (Bhujle *et al.,* 1979; Ambadkar and Chauhan, 1979b; Gupta and Maiti, 1986). Avian ovarian steroidogenesis has been described by Porter *et al.,* (1989) by using a three-cell model, which states that, the principle sources of progesterone, testosterone and estradiol are granulosa cells, theca interna and the theca externa cells respectively. In Chapter 1, variations in 3β-HSDH activities of extra-gonadal tissues are corroborated with variations in the activities of the same enzyme in the gonads over the reproductive cycle in the two species of Mynas.

17β-HSDH plays an essential role in the formation of active intracellular sex steroids and it catalyzes the interconversions between the low activity, neutral and phenolic 17-oxosteroids such as androstenedione and estrone, into highly active 17β-hydroxysteroids, such as testosterone and estradiol respectively (Baillie *et al.,* 1966; Andersson *et al.,* 1995; Moghrabi *et al.,* 1997; Lateef *et al.,* 1997; Dufort *et al.,* 1999) [Flow Chart 1]. According to Labrie *et al.,* (2000) in humans, seven types of 17β-HSDHs have been cloned which provide target cells with means of precisely controlling the intracellular concentration of each sex-steroid according to local needs. In the human ovary, 17β-HSDH is known to play a major role in the testosterone biosynthesis (Qin and Rosenfield, 2000), which facilitates the development of reproductive organ, and in testes in cellular differentiation during spermatogenesis and exhilaration of secondary sex characters and breeding behaviour. Furthermore, Type 5 17β-HSD, one of the seven types of 17β-HSD characterized in humans, catalyzes the transformation of 4-androstenedione (4-dione) into testosterone in the ovary and it also controls the optimal level of progesterone and sex steroids in the female reproductive tissues (Luu-The V *et al.*, 2001). 17β-HSDH is reported in the theca interna of normal growing follicles, atretic follicles and interstitial gland cells in the ovaries of Stork-billed Kingfisher (Bhujle and Nadkarni, 1978) and Blue Rock Pigeon (Kotak, 1979). In Chapter 2, relationships in variations in the 17β-HSDH activities of extra-gonadal and gonadal tissues are discussed and an attempt is being made to find out the involvement of extra-gonadal tissues in steroid metabolism.

3α-HSDH is an important multifunctional oxidoreductase capable of metabolizing steroid hormones, polycyclic aromatic hydrocarbons and prostaglandins (Stolz *et al.*, 1991; Usui *et al.*, 1994). Mammalian 3α-HSDHs inactivates circulating steroid hormones and in target tissues, regulates the occupancy of steroid hormone receptors (Penning *et al.*, 1997a). Reductive 3α-HSDH is known to terminate the action of potent androgen (e.g. 5α-dihydrotestorone) in target tissues (prostate) and oxidative 3α-HSDH isoforms are known to provide an alternative source of potent androgens by converting 3α-androstanediol to 5α-dihydrotestorone (Jin and Penning, 2001) [Flow Chart 1]. Mammalian 3α-HSDH inactivates circulating androgens, progestins and glucocorticoids and regulate access of steroid hormones to steroid hormone receptors in target tissues (Penning *et al.*, 1997b). The third Chapter deals with possible involvement of entero-hepatic circulation in recycling of steroid metabolites with that of bile acids and excretion of the same *via* biliary as well as nephric pathway. 3α-HSDH is reported to be involved in bile acid biosynthetic pathways and has been suggested to play an important role in net bile transport across the hepatocytes (Stolz *et al.*, 1989).

FLOW CHART – 1: PATHWAY OF STEROID BIOSYNTHESIS

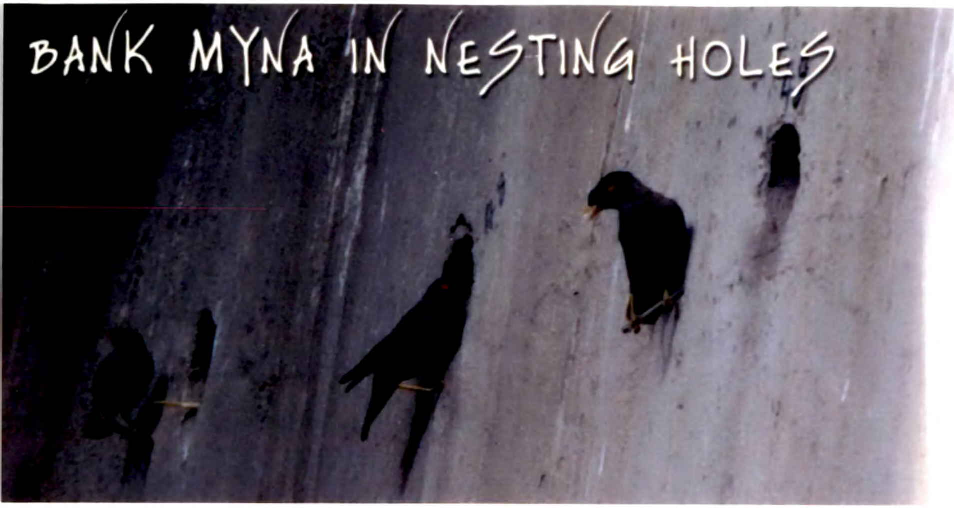


In Chapter 4, the histoenzymological alterations in the steroid dehydrogenases of gonadal and extra-gonadal tissues of the birds studied are correlated with plasma testosterone and plasma progesterone levels.

Having studied the changes in the hydroxysteroid dehydrogenase activities during the four phases of the reproductive cycle it was thought desirable to look into possible involvement of different components of lipids in the same tissues. Lipids are also known to serve as a source of energy store. It has been noted that the capability of birds for storing triglycerides as energy reserve exceeds that of other classes of vertebrates (Blem, 1976). Apart from the metabolic significance, lipids especially cholesterol positive lipids form the raw materials for steroidogenesis. Lofts and Murton (1973) have reported that the cyclic waxing and waning of cellular lipids, which though in itself is insignificant to implicate unequivocally a steroid synthesizing role, is a useful index of the functional state of the gonadal tissue. Chapter 5 deals with the variations in lipid accumulation in the gonadal and extra-gonadal tissues.

Concurrent histological observations are made in Chapter 6 regarding histomorphological changes in the ovary, testis, liver, intestine and kidney to reaffirm the changes occurring in these tissues during the four phases of the reproductive cycle.

BANK MYNA



BANK MYNA IN NESTING HOLES

BRAHMINY MYNA

BRAHMINY MYNA IN NESTING HOLE