CHAPTER : 1

HISTOMORPHOLOGICAL STUDY OF ANNUAL TESTICULAR CYCLE

The common Indian House Crow, Corvus splendens (Vieillot) needs no introduction. The crows, the elite of Corvidae, have been very closely associated with man from time immemorial. In spite of their familiarity and popularity, less is known about the physiology of reproduction. Studies on breeding habits of most Indian birds were started more than a century and a quarter ago. Jerdon (1862-1964), Hume (1873), Hume and Oates (1889-1892) and Baker (1932-1935) have reviewed the literature on the field studies regarding breeding seasons, nesting, nidification etc. of this and many other Indian birds. Dewar (1905, 1907), gave a very useful, more elaborate and original account of the breeding habits of crows, but he did not mention aspects like courtship, nest building, territory, incubation, feeding the young ones and the rate of mortality in young etc. Many ornithologists and naturalists (Foster, 1923; Whistler, 1923; Ali, 1926; Prater, 1926; Berriff, 1927; Inglis, 1931; Varghese, 1935; Babaseh, 1936; Rao, 1936; Amadon, 1944; Sen, 1947; Acharya, 1951; Jamal'Ara, 1954; Prasad, 1954, 1965; Paynter, 1961; Tiwari, 1962; Lamba, 1963, 1965a and b,
1969; Ali and Ripley, 1968-74), have written about the
nidification and some other aspects of breeding biology
of crows from time to time. Reports regarding the
nidification of this bird by Lamba (1963) and Holyoak
(1967) add to our knowledge of breeding biology of
crows. Prasad (1965) studied histologically the gonadal
cycles of crows of Varanasi, a place in north India.
His is the only histological work reported so far deal­
ing with seasonal changes in the gonads of crows of
India.

The breeding season of the crow (Corvus splendens),
seems to differ slightly in different parts of India
(Prasad, 1954, 1965; Lamba, 1963a). Hume, as early as
1889, stated that the breeding season par excellence
is June and July but an occasional nest may be found
earlier. A good number of crows may lay eggs in the
month of May. According to Dewar (1929), the breeding
season in the northern, western and central India is
June to August, most eggs being laid between June 10th
to 30th. Baker (1932-1935) has stated that over Eastern
Bengal, Bihar and Arakan, the normal breeding season
extends from March to April, but in Dacca and Mymensingh,
there are two well defined seasons—(1) December-January
and February in winter and (2) April, May and rarely
June in summer. In Ratnagiri and other parts of old Bombay Presidency, Davidson and Wenden (1878), Vidal (1880), and Davidson (1882, 1895), found that there were two similar seasons; the principle months being November and December and then again April and May.

From the literature cited above it is apparent that the breeding season of the house crow differs in different regions of varied environmental conditions. Considering such a state of knowledge it was thought desirable to re-investigate the reproductive cycles of Indian house crows in detail to gain better understanding about the environmental and certain endocrinological factors underlying the variations in annual breeding cycles of this species. The present chapter deals with the annual testicular cycle of the Indian house crows of Baroda. It is not uncommon in the birds of the tropical and equatorial regions (Misra, 1960-1961), to have "double gonadal cycles". Whether the double/single gonadal cycle is induced only under certain circumstances in certain areas or is it a regular feature of only a few populations in some of the areas? This is not properly understood. The study was aimed to find out probable answer to this question.
MATERIAL AND METHODS

The birds were collected every month from July 1970 to July 1975 by shooting with an air-gun in and around the University campus at Baroda (Long. 73° 13'E; Lat. 22° 18'N). 180 male crows (Corvus splendens), were studied. The birds were taken to the laboratory immediately after shooting. Birds were weighed and both the testes were collected. The glands were blotted free of tissue fluids and blood from the surface and then weighed accurately. The testes were fixed in Bouin's Fluid. Blocks were prepared by using routine alcohol grades, clearing the tissue in toluene and embedding in paraffin wax. Blocks were sectioned at 6 μ and stained with hematoxylin and eosin for microscopic examination. The following measurements were made: (1) diameter of at least 15 seminiferous tubules in transverse sections (2) the number of spermatogonial cells per cross-sectional area of seminiferous tubule for each bird (Table: 1.1). The average of not less than six birds per month was taken into account.

Annual cycle of testes volume was recorded (Fig. C). Testis volume was calculated using the formula for the volume of an ellipsoid: \( V = \frac{4}{3} \pi a^2 b \), where, \( a \) = one half the shorter diameter and, \( b \) = one half the longer diameter.
Male gonadal cycle of *Corvus splendens* at Baroda (Fig. A₁), the relation of stages of spermatogenesis with phases of testicular cycle (Fig. A₂), and seasonal variation in weight of the testes (mean monthly combined weight of both the testes) were observed. Head portion was exposed to see skull ossification and data from the juvenile crows were discarded. Relative numbers of interstitial cells were estimated according the method of Threadgold (1956b). For each sectioned testis a count was made of number of Leydig cells in five different sites selected at random. The mean number obtained from such counts was then multiplied by a factor \((a^2b)\), which was proportional to the volume of the testis, thus providing an index of the total number of Leydig cells in the testis.

**OBSERVATIONS AND DISCUSSION**

The system of dividing the spermatogenic cycle into histologic stages, adopted in the present study, is more or less similar to that employed by Wolfson (1942) for the Oregon Junco (*Junco oreganus*), by Bartholomew (1949), Davis and Davis (1954) for the house sparrow (*Passer domesticus*), by Johnston (1958) for California gull, and by Jonson (1961) for Mallard (*Anas platyrhynchos*), despite some difference in numbering of the stages.
Fig. A₁: Reproductive status (testes cycle) of *Corvus splendens* at Baroda, from June 1972 to July 1973.

Fig. A₂: Diagramatic representation of monthwise frequency of stages of spermatogenesis and distribution and overlapping nature of phases of reproductive cycle of *Corvus splendens*. 
Fig. B: Showing seasonal variation in the weight of testes. Each dot represents the mean monthly combined weight of both the testes.

* No less than five birds were considered in each month.
Fig. C: Annual variation in the testicular volume in the *Corvus splendens*, at Baroda.
Fig. D: Annual cycle in relative numbers of testicular interstitial cells (Leydig's cells) in the *Cervus elaphus*, at Baroda.
The histological changes in the average diameter of seminiferous tubules and average number of spermatogonial cells per seminiferous tubule in cross section.

<table>
<thead>
<tr>
<th>Month</th>
<th>Body weight (Gm.)</th>
<th>Testes weight (Gm.)</th>
<th>Average diameter of the seminiferous tubule in μ£</th>
<th>% increase, or decrease in seminiferous tubule in cross section</th>
<th>Average % increase, or decrease in number of spermatogonial cells per seminiferous tubule in cross section</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>282</td>
<td>0.0656</td>
<td>0.0233</td>
<td>104.40</td>
<td>22</td>
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<tr>
<td>February</td>
<td>266</td>
<td>0.0851</td>
<td>0.0313</td>
<td>114.80</td>
<td>30</td>
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<tr>
<td>March</td>
<td>258</td>
<td>0.1514</td>
<td>0.0537</td>
<td>119.33</td>
<td>32</td>
</tr>
<tr>
<td>April</td>
<td>268</td>
<td>0.2543</td>
<td>0.1190</td>
<td>160.20</td>
<td>38</td>
</tr>
<tr>
<td>May</td>
<td>248</td>
<td>0.3936</td>
<td>0.2600</td>
<td>198.96</td>
<td>48</td>
</tr>
<tr>
<td>June</td>
<td>255</td>
<td>1.9281</td>
<td>0.7350</td>
<td>236.24</td>
<td>51</td>
</tr>
<tr>
<td>July</td>
<td>269</td>
<td>1.5506</td>
<td>0.5760</td>
<td>228.94</td>
<td>45</td>
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<tr>
<td>August</td>
<td>252</td>
<td>0.3622</td>
<td>0.1382</td>
<td>152.25</td>
<td>41</td>
</tr>
<tr>
<td>September</td>
<td>271</td>
<td>0.0436</td>
<td>0.0161</td>
<td>147.32</td>
<td>10</td>
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<tr>
<td>October</td>
<td>280</td>
<td>0.0254</td>
<td>0.0091</td>
<td>143.70</td>
<td>12</td>
</tr>
<tr>
<td>November</td>
<td>286</td>
<td>0.0543</td>
<td>0.0189</td>
<td>180.41</td>
<td>12</td>
</tr>
<tr>
<td>December</td>
<td>291</td>
<td>0.0616</td>
<td>0.0212</td>
<td>098.83</td>
<td>19</td>
</tr>
</tbody>
</table>

* The average of the figures obtained for six adult birds per month.

* For each cross section fifteen different measurements were made.

* Shows % decrease.
Histologic stages 1 to 4 of the present system of study correspond to those of similar designation, used by the said workers. However, in order to indicate in greater detail the degree of activity achieved by testes in autumnal recrudescence, it was found convenient to subdivide the stage: 2, which corresponds to stages 2a and 2b of this study, and to stage 3 of Jonson (1961).

Stage: 4, of the present study corresponds to stage: 4 and 5 of Davis and Davis (1954), finally stages 5a and 5b of Wolfson (1942) and stage: 6 of Bartholomew (1949), on the basis of presence or absence of free, mature spermatozoa in the tubular lumina.

In establishing and interpreting histologic stages the report of Threadgold (1956) on the gonadal cycle of the male Jackdaw (Corvus monedulus) has been of special value.

Stage: 1: (Represented by a row of spermatogonia and rarely a few spermatocytes. Fig. 1a, 1b). The testes in this stage were of minimum size and the seminiferous tubules had the least possible diameter. The seminiferous tubules were separated by large areas of interstitial tissue (Fig. 8). Most of the seminiferous tubules depicted empty tubular lumina. Majority of the
seminiferous tubules were having a single peripheral row of spermatogonia but occasionally a few seminiferous tubules exhibited evidence of spermatogonial proliferation and centripetal localization of a few spermatocytes.

**Stage: 2:** (Represented by two or three rows of spermatocytes in addition to spermatogonia (Fig. 2a and 2b). Two sub stages: 2a (Fig. 2a) and 2b (Fig. 2b) were distinguished. In substage 2a, testis volume and tubule diameter showed a slight increase over stage 1 but the tubules remained separated by large areas of interstitial tissue. The majority of tubules contained two rows of spermatocytes. Little or no debris was present in the tubular lumina. With respect to tubular histology this substage is apparently identical to the "October ....... 2nd week" condition in the Jackdaw, as described by Threadgold (1956). In substage 2b, testis volume and tubule diameter showed further increase (Table: 1.1, Fig. C). Adjacent tubules were in close contact along their margins and areas of interstitial tissue got reduced to mere islands. Most of the tubules contained three more or less complete cellular rows comprising spermatogonia, primary spermatocytes and occasionally some secondary spermatocytes. Many of the primary
spermatocytes depicted the synapsis phase. This substage is similar to the "Early March" condition of the testis of Jackdaw (Threadgold, 1956).

Stage: 3: (Secondary spermatocytes very common, Fig.3). In this stage, most of the tubules possessed spermatocytes forming five or more rows above the basal row of spermatogonia. Cell proliferation was much in evidence. The tubules were further expanded in size over substage 2b (Fig. C). This stage is equivalent to Threadgold's "Late March" condition (1956), except that spermatids were not present.

Stage: 4: (Chiefly spermatids in formation. Fig. 4). This stage was characterized chiefly by the appearance of spermatids bordering the luminal side of the tubules. Spermatozoa were not present.

Stage: 5: (Showed full spermatogenic activity with many spermatozoa. Fig.5a, 5b). Testes in this stage were assignable to two different substages which were defined primarily on the basis of differences in distribution of spermatozoa within the tubules. In substage 5a (Fig.5a), there were no free spermatozoa present in the lumen and bundles of spermatozoa were not in the process of passing into the lumen. Otherwise substage 5a, was similar to
substage 5b. The latter was characterized by the occurrence of spermatogenesis as evidenced by the presence of free spermatozoa in the lumina of tubules. Tubular diameter did not differ significantly in the two substages.

Stage: 6 OR "R" Stage, OR Regression Stage: (Spent seminiferous tubules, lumen having cell debris, reduction in dimensions, Fig. 6a, 6b, 6c). The first indication of regression was simultaneous casting off of any remaining spermatozoa along with the other epithelial elements of the tubules as a mass of detritus (Fig. 6a, 6b). Such cell debris with spermatozoa were present in the epididymis also (Fig. 6d). During later periods of this stage, the seminiferous tubules contained small numbers of primary and secondary spermatocytes along with cellular detritus. At the beginning of regression, which should not be confused with substage 5b, whole bundles of spermatozoa along with other cell-type and cellular debris apparently pass en mass into the lumen.

Phases of Reproductive Cycle: The reproductive organs of the Indian house crow (Corvus splendens) were observed to undergo marked annual variations in size and functional activity like most of the other birds. The observations on the histology, weight and volume changes
in testes indicated the occurrence of only one full testicular cycle in a twelve-month period and was found to be a constant feature for the population of house crows (Corvus splendens) in Baroda.

Recrudescence of the testes began around late October and November continued through March, April and early May. The entire period of recrudescence involved approximately about seven months. Increase in testis weight was rapid (Fig. B) from the start of recrudescence but it declined in August and September. In April, however, there was much variation in testis weight, associated with variation in the time of onset of recrudescence for individual males. Testicular weight registered a progressive increase beginning in late May and reaching a high peak in June which was maintained at that level upto about early July. The highest testicular weight (1.9375 gms.) was recorded in June. Testes began to regress in late July and the process continued through August and September (Fig. B and C). A very small number of crows could maintain spermatogenesis around this time. While both testicular weight and size were decreasing, rarely any sample showed spermatozoa in the seminiferous tubules. Regression of testis, unlike recrudescence,
was comparatively a very rapid process and most of the birds had reached fully regressed condition (Stage: R, OR 6) by the middle of August.

Monthwise distribution of stages of spermatogenesis is given in Fig. A2. It is evident that Stage: 1, was found in the month of August, September and October. The samples of testes collected during August and September were generally having row of spermatogonial cells but having no spermatocytes, whereas, in the samples of late October and November a few spermatocytes in addition to spermatogonial cells were quite evident. Thus, it can be said that actual recrudescence begins in November. Recrudescence proceeded through Stages: 2, 3, 4 and 5a finally reaching fully active spermatogenic phase within approximately a seven months' period. Stage: 2, can be traced out in late October, November, December, January and February but frequency of occurrence was higher in the month of January. While Stage: 3 appeared in November and continued through April having high frequency in February. In March, April and early May, Stage: 4 was evident having higher frequency in late April and early May. Stage: 5a and 5b were common in late May, June and early July. From these data, it is evident that Stages: 4 and 5a and 5b occur through approximately two months each.
Spermatogenesis was completely over by the middle of July and then the Stage: R (that is regression) set in by late July and extended up to September. The frequency of occurrence of regression was highest in September. Regressed condition lasted for only two and a half months. It was observed that the recrudescence took much longer time while regression was abrupt and of very short duration and perhaps during the intervening period it developed refractoriness. Thus, it could be assumed that the late regressive phase (August and September) might represent the refractory period.

The growth of the seminiferous tubule was maximum in breeding phase (mean value of the diameter in this phase was 229.5 μ) and it suddenly dropped by about 321.09% in the regressive phase, reaching the mean diameter of 54.420 μ. The mean values of seminiferous tubules diameter in preparatory phase and progressive phase were 99.61 μ and 166.16 μ respectively. It is evident from the table: 1.1 that there was an increase of 83.09% in preparatory phase over the mean tubule-diameter value of regressive phase. A further increase of 66.81% in the mean tubule diameter was noticed during the progressive phase over that of the preparatory phase. Growth was maximum from March to April amounting to a 51.009%
increase. On the other hand, a sudden drop was evident from late July to August accounting a 338.162% decrease. This significant decrease in the month of late July continued into September.

Based on these observations, the annual reproductive cycle of the Corvus splendens inhabiting Baroda, could be divided into four phases (Fig. A2). Following three criteria were used in determining the phase of the reproductive cycle of male Corvus splendens in the present study:

1. Weight and volume changes of testes.
2. Spermatogenic activity as determined by histological analysis and
3. Cell count per seminiferous tubule and changes in diameter of seminiferous tubules during the annual reproductive cycle.

(A) Regressive Phase: (Late July, August, September and early October). Involved casting off of a large number of spermatozoa as well as degenerating cells into the tubular lumina, (Fig. 6a,b and c). Registered minimum weight and volume of the testes and reduction in seminiferous tubule diameter.

(B) Preparatory Phase: (Late October, November, December, January and February). Seminiferous tubules
showed two or three rows comprising of spermatogonia, primary spermatocytes and secondary spermatocytes (Figs. 2a, 2b and 3). During this phase progressive increase in weight and volume of testes was observed.

(C) **Progressive Phase**: (March, April and early May). Depicted presence of secondary spermatocytes, spermatids and rarely a few spermatozoa. Progressive increase in weight and volume of testes was maintained (Figs. 3, 4).

(D) **Reproductive Phase**: (Late May, June, early July). Full spermatogenic activity with many spermatozoa was apparent (Figs. 5a, 5b and 7). Maximum weight and volume of the testes were recorded.

**Leydig Cell Cycle**: Seasonal variation in the number of Leydig cells is shown in Fig. D. Cyclic variation in the number of Leydig cells runs parallel to those of testicular volume (Fig. C) as well as weight (Fig. B). An increase in number of cells was first noticed in mid-October (Fig. 8) and, thereafter, a further gradual increase was recorded through November, December, January and February. In the month of March the increase noted was quite abrupt (49.012%), as compared to the value obtained in February. Later, the
rate of increase more or less plateaued off until May. The maximum number of Leydig cells could be observed during June and early July (Fig. 7). A sharp decline in the relative number of cells was recorded to begin by early August which continued progressively through September and early October. Taking into account the above mentioned facts, it could be concluded that, the Indian house crow (Corvus splendens) depicts a single-peaked cycle of the interstitial cell number. Such a unimodal cycle of house crows probably necessitates essentially a region-wise/population-wise distinction between single and multi-brooded species. The latter obviously requiring a longer period of testicular activity where partial regression in testis volume, histologic stage and interstitial cell numbers during a breeding season probably occurs giving the cycle its seemingly two-peaked form. A comparable unimodal pattern has been reported in the cycles of testis volume, seminiferous tubule diameter and interstitial cell number in Jackdaw (Threadgold, 1960). Whether a true bimodal pattern in histologic stages, testicular volume and interstitial cell numbers occurs or not in the breeding seasons of house crows (Corvus splendens) and other Corvids found in India is not known so far. Studies of Threadgold
(1960) and Selander and Hauser (1965) in four populations of house sparrow and Great tailed Grackle respectively revealed a bimodal pattern of testicular activity in those species. As an explanation for the bimodal pattern, Threadgold (1960) has offered two possibilities: First, the transfer to the seminal vesicles of spermatozoa produced in the Spring, or their use in copulation, may cause a reduction in the volume of the seminiferous tubules and hence, of the testis as a whole. Secondly, sex hormones produced earlier in the season may inhibit secretion of follicle stimulating hormones by the pituitary, with a consequent reduction in the rate of spermatogenesis and testicular volume. It may be said in the case of Indian house crow (Corvus splendens) that the regression of testis in August and September could be attributed to both the possibilities suggested by Threadgold (1960). Though Threadgold's postulated mechanism of an androgen feedback effect on the pituitary is a plausible explanation, yet, we believe that it may be worthwhile to consider another possible factor. It was observed in the case of Corvus splendens that the major nesting effort of the females was evident in the month of May (and fewer females started their nesting activities in early June; occasionally in August too, only
in bixeric regime populations of crows) (Lamba, 1963; 1965, 1965b and 1969). Moreover, seasonal histologic study of crows' ovaries (Chapter: 2), revealed that a very quick and maximum ovarian growth occurred around the middle of June, followed soon by actual ovulation. Duration of ovarian growth culminating into ovulation was only of a very short duration (7 - 10 Days approx), whereas, the rate of testicular development was comparatively slow. It has also been noticed that complete regression of testes comparatively took longer time than the time taken by ovary to regress. There are, therefore, relatively fewer opportunities for males to mate in late July or early August than in early and mid-June. Thus, it seems possible that, as a result of a diminished amount of flow of stimuli provided by the females in late July and early August, there was a slight diminution in the rate of production of gonadotropins by the pituitaries of adult males. There was not only an apparent slowing of the rate of spermatogenesis but possibly a decrease in the rate of production of androgen by the testes. It could be said for the populations of crows in other bixeric regions that the second peak of the breeding activity in November and December could possibly be triggered on by the second nesting effort.
exhibited by the female crows of those regions. The suggestion regarding maintenance of testicular activity depending upon stimulation from the sexually active females is in accordance with the observations of Burger (1953) on the effect of the presence of females on gonadal development in male birds.
EXPLANATION TO FIGURES

PLATE: I

Fig. 1a to 5a. Photomicrographs of the T.S. of testes of Corvus splendens, depicting the stages as indicated against figs. 1a to 5a.

Fig. 1a. Stage - 1a, Resting spermatogonia only. X 550.

Fig. 1b. Stage - 1b, Spermatogonia dividing, but only a few spermatocytes present. X 300.

Fig. 2a. Stage - 2a, Many primary spermatocytes present, forming two tiers of cells. X 550.

Fig. 2b. Stage - 2b, Many primary spermatocytes and very few secondary spermatocytes present, forming three tiers of cells. X 550.

Fig. 3. Stage - 3, Secondary spermatocytes very common. Note increase in number of secondary spermatocytes, more than three tiers of cells. X 250.

Fig. 3a. A portion of fig. 3 magnified. X 500

Fig. 4. Stage - 4, Spermatids - Primary as well as secondary - but no spermatozoa. Note centripetal variation in nuclear shape and size. X 300.

Fig. 5a. Stage - 5a, Full spermatogenic activity with many spermatozoa attached. X 250.
PLATE : II

Fig. 5b. Stage - S, Note occurrence of spermeation evinced by the presence of free spermatozoa in the lumen of the tubule. X 250.

Fig. 5c. Content of seminiferous tubule (Stage-S). Note free spermatozoa with some other discarded cells. X 500.

Fig. 6a. Stage - R, Note the cell debris in the tubular lumen. Just the beginning of regression depicted by the detachment of inner cells. X 250.

Fig. 6b. Stage - R, Note the reduction in the tubular diameter and reduction in cell height. Single cell-layer remaining. Cell-debris in process of passing out and dissolution seen in the lumina of tubules. X 250.

Fig. 6c. Stage - R, Tubular lumina free from any cell-debris. Note further reduction in diameter and prominent but small size Leydig Leydig cells. X 350.

Fig. 6d. Content of Seminiferous tubule (Stage-R). Note the cell-debris having no spermatozoa. X 500.

PLATE : III

Fig. 6e. T.S. of epididymis, from a specimen with regressed testes. Note smaller diameter and
absence of any seminiferous tubule elements (cellular). X200

Fig. 6f. T.S. of testes (from reproductive phase).
Note cell-debris along with spermatozoa in lumina. X 200.

Fig. 7. T.S. of testes (from non-breeding phase) to show prominent interstitial cells in clusters.
Note the diameter of the tubules and increasing strands of interstitium. X 300.

Fig. 8. T.S. of testes (from reproduction phase) to show empty, vaculated and crumpled interstitial cells having small dot like nuclei. Interstitium sandwitched in the form of thin strends, between enlarged seminiferous tubules. X 150.