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The Development of Duck Tests

G. A. R. Kamar and K. A. Yamani

Faculty of Agriculture, Cairo University, Cairo, Egypt.

FIVE MALES of Pekin ducks, at hatch and of1,2,3,4,5,6,9,12 months of age and 5 males of 12 months of age of Rouen and Sudani ducks were available. Morphological and histological examination were done for the testes of these birds.

It was found that the developmental changes of the testes, seminiferous tubules and spermatogenesis in this study can be divided into three developmetal stages. The first stage, during the first four months of age, is characterized by slight increase in testes size, tubule diameter and cell number. The spermatogonial cells proliferated by the mitotic divisions into two layers. Primary and secondary spermatocytes were formed adjacent to the layers of spermatogonial cells, and also the spermatids were fomed. The second stage is characterized by the highest increase in testes size, cells number and tubules diameter accompanied by the formation of the largest numbers of spermatids and spermatozoa. This stage extended from the forth to ninth month of age when sexual maturity is attained. The third stage takes presedence afterwards where slight changes occurred in testes size, cells number and tubules diameter. The Sudani drake being heavier in body weight, was of lighter testes and less reproductive activity. On the contrary was the Rouen, while the Pekin draks was moderate in this respect.

In general, the development of the seminiferous tubules and the component parts of the testes in birds can be distinguished into four stages. The first is during the first five weeks of age when the organization of tubules and the multiplication of the basal layer of cells result in a gradual increase in the diameter of the tubules. The second is from six to nine weeks of age when primary spermatocytes begin to appear then. The third is at the tenth week of age when the secondary spermatocytes begin to appear as produced by the meitotic division of the primary spermacytes. On the twelfth week the fourth stage occurs as spematids begin to appear in the siminiferous tubules. By the twentinth week of age, spermatids takes precedence in all the seminiferous tubules, accompanied by a great growth in the length of tubules thus increasing the capacity of the testes to produce mature spermatozoa (Kumaran and Turner, 1949 and Kamar, 1960).

Material and Methods

Five male Pekin duck at hatch, and of 1,2,3,4,5,6,9, and 12 months of age were used. Ducks were hatched at one month interval and at the end of the year all the ages were available. The slaughter test was done for one individual from each age each 3 days. Testes for histological exminations were preserved in 5% formalin. At 12 months of age five males of each of the Rouen, Pekin and Sudani ducks (Native ducks) were slaughtered. Absolute and relative weights were recorded for the reproduction organs. All the birds were reared, housed, managed and fed alike.

Samples of testes were fixed, washed, dehydrated, cleared and embeded in parafin. Blocks of both right and left testes were sectioned at 10 microns. The sections were stained by the heamatoxylin and eosin method. The number of seminiferous tubules was counted per square centimeter. The diameters of seminiferous tubules were measured for circular tubules. The diameters of the shorter and longer axes of oblique tubules were measured. The number of tubules were determined in 100 separate microscopical fields from 10 slides for each right and left testes of each of 5 individuals summing up 1000 counts for each age. The diameter was measured for five tubules in each field summing up 5000 measurements for each age.

Results and Discussion

Morphological studies

Testes increased, gradually, in absolute and relative weight with the advancement of age up to the ninth month, yet a remarkable decline was observed afterwards (Table 1). Age differences in testes relative weights were highly significant. At four months, the testes represent a very small absolute and relative weight, then a rapid increase was observed on the fifth month of age on subsequent ages, reaching the maximum weight on the ninth month.

The vas difference and penis showed almost the same trend in absolute weight like the testes, except that they continued the increase until 12 month. The relative weight of penis decreased until 4 months then increased rapidly afterwards. During the prepubertal period, the penis is of no vital physiological function, while after 4 months when sexual maturity is supposed to be attained it in enlarged in size to be used for matings. Age differences for the relative penis weight were highly significant.

The testes of the pekin and Rouen were larger in absolute and relative weights than those of the Sudani (Table 2). The testes of lighter breeds such as Pekin and Rouen, normally, exceed that of the heavier one such as Sudani as found also in chickens (Kamar and Mostageer, 1960). The vas deference and its absolute and relative weights of the penis followed the same breed differences of testes. Breed differences in penis relative weight were significant.

Histological description

At hatch

Seminiferous tubules were lined with either one or two layers of spermatogenic epithelium next to the basement membrane of the tubules. The spermatogonial cells were few and the layers were not complete. The seminiferous tubules showed evidence of tortousity. In this early stage of life, no spermatocytes could be easily distinguished. The lumen was observed in some tubules. The seminiferous tubules were surrounded by abundant connective tissue and a small number of interstitial cells.

TABLE 1. Average absolute and relative weights of male reproductive organs at different ages

Items		At hatch	Age in months							
			1	2	3	4	5	6	9	12
Testsx	Wt.	0.003	0.010	0.034	0.126	0.262	9.070	45.130	118.580	44.300
	Rel.Wt.	0.0076	0.0052	0.0071	0.0125	0.0151	0.3268	1.8749	4.9470	2.2139
Vas diffe-	Wt.	0.010	0.021	0.038	0.122	0.125	0.232	0.800	1.000	1.290
rences	Rel. Wt.	0.0255	0.0109	0.0079	0.0121	0.0072	0.0109	0.0323	0.00421	0.667
Penisxx	Wt.	0.020	0.032	0.042	0.054	0.060	0.960	4.250	4.930	5.570
	Rel.Wt.	0.0510	0.0165	0.0088	0.0054	0.0035	0.0452	0.1766	0.2057	0.2784
Total	Wt.	0.033	0.063	0.114	0.230	0.447	10.262	50.180	124.510	51.160
	Rel.Wt.	0.0841	0.0326	0.0238	0.0300	0.0258	0.3829	2.0838	5.5590	2.5590

X F values : Bewteen Ages=69.15xx (Highly significant).
XX F values : Between Ages=42.44xx (Highly significant).

TABLE 2. **Average absolute and relative weights in male reproductive organs of different breeds.**

Items	Pekins		Rouen		Sudani	
	Wt.	Rel.Wt	Wt	Rel.Wt	Wt.	Rle.Wt
xTestes	44.30	2.2139	39.32	2.6354	11.36	0.4727
Vas differences	11.29	0.0667	0.92	0.0617	0.83	0.0345
xxPenis	5.57	0.2784	4.09	0.2741	4.42	0.1839
Total	51.16	2.5590	44.33	2.9171	16.61	0.6911

One month

No obvious changes were observed in this stage of age compared with the previous one. The spermatogonial cells were still few and scattered in one or two layers following the basement membrance. The seminiferous tubules were markedly coiled in some tubules while the others showed less evidence of tortuosity. The lumen was observed in a clear condition in more tubules. Abundant stroma were seen full of interstitial cells.

Two months

At this age, the spermatogonial cells increased in number in comparison with the previous ages. The earliest of the germ cells, the spermatogonia, rest upon the basal lamina, while the later stages are found at successively higheir levels in the epithelium. Prominent primary spermatocytes in large size were easily observed as the mitotic activity took place. Small lumina were not clear as those previously described in the previous age. This may be due to the increased activity of cell division. The seminiferous tubules were still showing slight convolutions. The connective tissue increased in size. Numerous interestitial cells were seen but they were of small size.

Three months

Several layers of cells clearly lined the seminiferous tubules. The spermatogonial cells continued several mitotic divisions with the production of several layers of spermatogonia. The proliferation was towards the lumen. The primary spermatocytes underwent the reduction division, *i.e.* meiosis, forming smaller secondary spermatocytes in small numbers at the middle of the lumen. The lumina were abundant and larger in size in most of the tubules. Almost half of the seminiferous tubules were convoluted whiile the other were in spherical or oval shape indicating slight development in the tubules length. The stroma represented a larger size of testes tissue in which numerus interstitial cells were scattered.

Four months

The spermatogonial cells, which were larger in size and number, lined the seminiferous tubules with two stratified compacted layers next the basement membrane. The mitotic and meiosis divisions continued and the primary and secondary spermatocytes increased in number and size. The secondary spermatocytes underwent mitotic division and began to produce spermatids of a rather small number and size. Each of the relatively small spherical or polygonal spermatids resulting from divsion of the secondary spermatocytes has a nucleus 5 to 6 microns in diameter with pale-staining finely granular chromatin and several darker chromatin masses. A small Golgi apparatus can be seen the cytoplasm. The secondary spermatocytes underwent mitotic division and began to produce spermatids of a rather small number and size. The spermatids were scattered in the middle of the lumina mixed with secondary spermatocytes. Well-formed lumina were now seen in all the tubules and this was full of spermatids. The seminiferous tubules were mostly of spherical shape but more convoluted than the previous age. The stroma increased in size and full most of the intertubular spaces. Also, the interstitial cells increased in size and number.

Five months

Cells of different types increased greatly in number. Also, the spermatids in large numbers and spermatozoa in few numbers were filling the well-formed large lumen. An increase in seminiferous tubules size were observed. Small vacuoles between cell layers were observed as an indication of the beginning of the formation of Sertoli cells. The connective tissue stroma were still fibrous but abundant, containing the increasing number of interstitial cells, which were more than before.

Six months

The spermatogenic activity was higher at this age than all the previous ages. The continual proliferative activity of the seminiferous epithelium is confined to the spermatogonia and spermatocytes near the base, and the neogenesis of succeeding generations of cells in this region displaces the more mature forms to higher levels as they differentiate, so that, as mature spermatozoa, they come to border directly upon the lumen. All the stages of spermatogenesis took place in more layers. The spermatogonia, primary and secondary spermatocytes and the spermatids were greater in numbers and stratified in several layers between which the Sertoli cells existed. Large numbers of Spermatids were present, scattered in clusters and accompanied by moderate numbers of spermatozoa, extending towards the lumen, and clumping around the scattered Sertoli cells. Also, the lumen contained some loose spermatozoa. The first sign of differentiation of a specific component of the spermatozoa is the appearance of several granules within the Golgi apparatus. The tubules were now greatly convoluted indicating rapid growth in tule length. The connective tissue stroma was now well formed and abundant. The cells of leydingh which were now formed, increased in number and occupied most of the stroma. Each Sertoli cell is fixed to the basal lamina of the seminiferous epithelium and has

its nucleus and celll centre situated near it. The remainder of the cell forms an extraordinary elaborate system to this processes that extend upward to the free surface, surrounding the spermatogenic cells and filling the interstecia.

Nine months

No further changes occurred at this age, increased numbers of spermatozoa were observed. The seminiferous tubules were slightly more convoluted as development progresses the several separate granules coalesce into a single large gulobule, the scrosomal granule, contained within a membrane bounded acrosomal vesicle or vacuole. This becomes adherent to the outer aspect of the neuclear envelope. This forms the tip of the sperm nucleus. The Golgi apparatus remains closely associated with the surface of the acrosomal vesicle which enlarges with the advancement of age.

12 *months (Pekin)*

This age was characterized by high spermatogenc activity. All the stages of spermatogenesis in greater numbers were observed consisting several layers of cells. Waves of mature spermatozoa were filling most of the lumen. The tubules were greatly convoluted. The stroma was more abundant than the previous ages but occupied less size of the testicular tissue. Well formed, numerous cells of Leydis were filling the intertubular spaces. The seminiferous tubules are lined by a complex stratified epithelium composed of two major categoris of cells: supporting cells and spermatogenic cells. The supporting elements are of a single kind, the Sertoli cells, while the spermaogenic cell include several morpholgically distinguishable types : spermatogonia, primazy spermatocytes, secondary spermatocytes, spermatids and spermatozoa.

Overlaping occurs between the different phases in sections from differen individuals at the same age. Cells of later stages may be observed at earlier ages in the testes of certain individuals, and some sections depart from the general average of all the individuals.

12 *months (Rouen)*

The spermatogenic activity of Rouem's testicular tisue was the highest among the studied breeds of the same age. Numerous cells, were stratified in several layers representing all the stages of spermagensis. However the spermatozoa occupied less size in the lumen in comparison to the previous breed.

12 *months (Sudani)*

The spermatogenesis process was of less active divisions in comparison with that of the Pekin or the Rouen resulting in less numbers of cells of different stages. Although all the stages of spermatoagenesis were observed, the spermatogonial cells, the primary and secondary spermatocytes were less in number and layers than the other two breeds. The layers were not complete, and the cells were scattering and not compact. The lumen was smaller in size than the other breeds. The seminiferous tubules showed less convolution. The stroma were fibrous and there were few numbers of interstitial cells.

Age differences

The number of tubules per unit area were more or less the same in the first three age groups (Table 3). The diameters of the seminiferous tubules, also showed slight changes throughout the same period. Afterwards, a drop in the number of tubles per unit of area, beside a remarkable increase in the diameters of these tubules were observed. The previous observations were associated with the rapid increase in tests size. It seems that the utmost spermatogenic activity and the highest development of the seminiferous tubules took place at five months of age.

TABLE 3. **Histological changes in testes tissues with age.**

Age in months	Average rel.Wt.and testes (1)	Absolue of pair of	No.of tubules per cm² of T.S.	Average diameter of tubule	Average No of cells/ tubule sect. (4)
	Wt.	Rel.Wt	(2)	(3)	
at hatch	0.003	0.0076	26449	44	19
1	0.010	0.0052	23770	646	30
2	0.034	0.0071	26784	46	32
3	0.126	0.0125	28123	48	81
4	0.266	0.0151	20454	88	132
5	9.070	0.3268	15903	96	155
6	45.130	1.8749	4185	160	434
9	118.580	4.9470	2845	194	402
12	44.300	212.39	2678	198	554

(1) Average weight of the pair of testes from one mole obtained by dividing the total weight on right and left testes from five birds by five.
(2) Average number in 1000 microscopic fields for each age group.
(3) Average of 5000 measurements ofr each age group.
(4) Average of 1000 seminiferous tubules for each age group.

The cells increased in number steadily but within a narrow limit during the first two months. Through this period of age only spermatogonial cells and primary spermatocytes were observed. After that a slight increase in cell number occurred on the third and fourth month of age. Most of the changes in cells were due to the development and not due to the increase in numbe as most of spermatogenic stages were found at this age including the stages from the spermatogonia until spermatids. Rapid increase was observed on subsquent ages as the maturation of the spermatids produced great numbers of spermatozoa and the cells of each stage increased in numbers and layers. As

sexual maturity is attained by the appearance of the first spermatozoa in the seminiferous tubules (Kamar, 1960), it is suggested that sexual maturity in ducks begins at about five months of age. The number of cells in seminiferous tubules increased on subsequent ages of the study due to the increased spermatogenic activity of the drakes.

The developmental changes of the testes, seminiferous tubules and spermatogenesis in this study can be divided into three developmental stages, whilest it is divided into four phases in mammals (Roosen-Runge, 1962). The first stage, during the first four months of age, is characterized by slight increase in tubule diameter and cell number. The spermatogonial cells proliferated by the mitotic divisions into two layers. Primary and secondary spermatocytes were formed adjacent to the layers of spermatogonial cells, and also the spermatids were formed. In this period from the appearance of the proacrosomal granules, the development of a hemispherical acorosmal granule fixed to the nucleus and the increase of the acrosomal vesicle layer, forming a thin fold that spread outwards from the pole of the nucleus, to cover its entire anterior half as a membrandes head cap. The second stage is characterized by the highest increase in testes, cell numbers and tubules diameter accompanied by the formation of the largest number of spermatids and spermatozoa. This stage extended from the fourth to fifth month of age. In this stage, there is a redistribution of the acrosomal substance, a condensation of the nucleoplasm and an elongation of the spermatid. Acrosome substance spreads to form a fine tip comprising the head cap. The spermatid nucleus becomes a spindled shape in this phase. The third stage takes presedence from the sixth month and onwards, where slight changes occurred in testes, cells number and tubules diameter. During this maturation takes palce there further changes in the acrosome which ends by its final shape. The dense granules in the nucleus become coarser, increasing in size at the expense of the intervening spaces and the nucleus take the spindle klike shape. The tail at this period is long and have an axial filament complex. These three stages of testicular devlopment were also suggested by (Kamar, (1960) working on Fayoumi cockrels. However, Kamaran and Turner (1949) suggested four developmental stages in testicular tissue growth in White Plymouth Rock cockerels.

Breed differences

The Rouen drakes were of the highest average diameter of the seminiferous tubules and the number of cells per tubule. The Sudani drakes were the lowest, meanwhile the Pekin drakes were relatively high in the previous resp ect. The Rouen drake was the lowest and the Sudani was the highest, while the Pekin was moderate when the number of tubules per a unit of area was concerned (Table 4). This may suggest higher spermatoenic activity in Rouen drakes than Pekin and Sudani ones. This may be attributed to the testes size which followed the same breed differences. This trend of variation between breeds in the previous items was also observed in semen production as found by Kamar (1962) when the Rouen drake produced the highest semen qualities and quantities followed by the Pekin then the Sudani.

TABLE 4. Histological studies in different testes estimates of Pekin Rouen and Sudani ducks (Average of 5 males at each age).

Breeds	Average weight and relative weight of pair of tests		Number of tubules per cm^2 of T.S.	Average diameter of tubules	Average number of cells per tubules section
	Wt.	Rel. Wt.			
Pekins	44.30	2.2139	2.678	189	554
Rouen	39.32	2.6354	1.071	232	647
Sudani	11.36	0.4727	5.098	174	401

The interstitial tissue

They are irregularly polyhedral, 14 to 21 microns in diameter and occurs in isolated clusters or in rows along the small blood vessels. They are found in all the ages, but they are more abundant in the aged. They vary in shape and size. They have the staining properties of protein, having little affinity for the histological stains used in this study. Their nucleus are relatively large and spherical. The cytoplasm is strongly acidophilic. Occasionally, free of granules interstitial cells are observed, which may be considered as immature forms.

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نمو الخصية فى ذكور البط

محمد جمال الدين قمر و كمال عرفه يمنى
كلية الزراعة جامعة القاهرة و جامعة الزقازيق

كان النمو فى وزن الخصية والأنابيب المنوية والخلايا البينية وتكوين الحيوانات المنوية منقسما الى ٣ مراحل ـ الأولى خلال الأربع شهور الأولى من العمر وكان النمو فيها بطيئا والثانية كان النمو فيها سريعا وصاحبه النضج الجنسى وتكوين الحيوانات المنوية وذلك خلال المدة من ٤ ـ ٩ شهور من العمر والمرحلة الثالثة ثبت فيها النمو على ما وصل اليه فى المرحلة السابقة واستمرت الى عمر ١٢ شهر .