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TESTIS HISTOSTRUCTRURE DYNAMICS DURING QUAIL (*Coturnix coturnix*) SPERMATOGENESIS

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Abstract

Male sex cells are unique objects for scientific research in the field of genetics and physiology and in the study of the development biological basis in animal husbandry. Maturation and differentiation processes in male animals and birds germ cells are of great interest for comparative embryology, developmental biology, medicine and biotechnology. Quails characterized by early puberty and a short generation period are perspective for these experimental works. The greatest interest is the use of spermatogonia, the testes stem cells which are currently being actively studied as promising targets for the introduction of recombinant DNA in obtaining transgenic individuals. However, the morphology of germ cells from male poultry in their formation process is not fully covered. For the first time, we describe in detail the histological features of spermatogenical quail epithelium tissue at different stages of spermatogenesis and the dynamics of spermatogonial testis cells populations in this study. The aim of the study was to identify age-related features of spermatogenesis associated with the dynamics of the different cell type development in the epithelial spermatogenous layer of the seminiferous tubules in quail. For this, we examined the histological structure of the testes in quail (*Coturnix coturnix*) of the Estonian breed at the age of 1, 2, 3, 4, 5, 6, 12, and 24 weeks. In each age group, there were 10 males. Testis tissue was fixed in Bouins's solution, dehydrated in alcohols of increasing concentration and embedded in paraffin. Five to six micron histological sections were stained with hematoxylin-eosin. The composition of spermatogenic cells and their ratio in the seminiferous tubules was investigated. At least 30 seminiferous tubules were examined from each male. The diameter of the seminiferous tubules in the quail testes changed during ontogenesis and at the age of 1, 2, 3, 4, 5, 6, 12 and 24 weeks reaching 42±1, 71±2, 91±2, 117±2, 237±4, 278±5, 28 ±7 and 291±6 μm, respectively. Sertoli cells and generative cells were parts of cell population of the quail seminiferous tubules at different stages of differentiation, i.e. spermatogonia, spermatocytes, spermatids and sperm cell maturation. The number of spermatogenic cells inside the seminiferous tubules increased with age ($p < 0.01$) and was 18±1, 24±1, 58±4, 80±6, 249±16, 587±34, 658±24 and 540±41 in quails aged 1, 2, 3, 4, 5, 6, 12 and 24 weeks, respectively. In 1-week aged quails, Sertoli cells dominate in seminiferous tubules (12±1 per seminiferous tubule) while spermatogonia are few, 1 to 4 cells per tubule. The number of spermatogonia increases with age. The percentage of spermatogonia is maximum in 3-week aged birds, 76±2 % of the total number of spermatogenic cells. In 4-week aged quails, primary and secondary spermatocytes are visualized in the seminiferous tubules, and from week 5 spermatids are found. At the age of 5 weeks, we detected single spermatozoa, the number of which increased in the quail semen tubules by the 6-week age. Thus, the quails' age from 1 to 3 weeks is optimal for manipulating spermatogonia as targets for introducing recombinant DNA in order to obtain transgenic offspring or biological material to preserve the genetic resources of farm birds in cryobanks.

Keywords: quail, testes, Sertoli cells, spermatogenesis, spermatogonia

The study of maturation and differentiation of male germ cells of ani-

mals and birds is of great interest for comparative embryology, developmental biology, medicine and biotechnology in general. This is especially true for spermatogonia as the precursors of sperm, the mature germ cells. Spermatogonia are promising targets to produce transgenic individuals via the introduction of recombinant DNA [1]. Such technology involves the isolation and in vitro transformation of spermatogonia with their subsequent transplantation into the testes of male recipients in which their own spermatogenesis is previously blocked [2, 3]. Transplanted spermatogonia subsequently differentiate into sperm cells, which are used to produce transgenic offspring. Such a possibility of obtaining chimeric and genetically modified individuals was shown in a number of works on laboratory animals [4, 5], pigs [6, 7], sheep [8], as well as on roosters [9, 10]. Male gonad cells are also used as valuable genetic material for creating cryobanks as part of the conservation and maintenance of the gene pool of valuable breeds of animals and birds [11, 12].

Unlike mammals, in birds, including quails, testes remain in the abdominal cavity (in the place of their development) throughout life [13, 14]. Features of the genital organs of chickens, turkeys, ducks, and quails are rather deeply described in terms of anatomy, while the morphology of the cells of male poultry is described incompletely [15].

In birds, as in mammals, spermatogenesis is a long process of the gradual transformation of germ cells into spermatozoa within the boundaries of the seminiferous tubules of the testis and includes three successive stages, the spermatocytogenesis, spermatidogenesis, and spermiogenesis. The physiological and anatomical characteristics associated with spermatogenesis in birds are the subject of extensive research [16, 17]. During spermatogenesis, cell proliferation occurs with repeated mitotic divisions, duplication of chromosomes, meiotic cell division, etc. for the further development of haploid spermatids and their subsequent differentiation into mature sperm [18].

In the present paper, the histological features of the tissue of the spermatogenic quail epithelium at various stages of spermatogenesis were investigated for the first time in detail with characterization of the dynamics of the spermatogenic testis cell populations.

The purpose was to reveal the age-related features of spermatogenesis in quail in connection with the dynamics of the development of different types of cells of the epithelial spermatogenic layer of the seminiferous tubules.

Tehcniques. In the experiments, Estonian male quail (*Coturnix coturnix*) at the age of 1, 2, 3, 4, 5, 6, 12, and 24 weeks were used (groups of eight age categories, 10 animals per group). The biomaterial was the testes collected during slaughter.

The selected testis tissues were fixed for 48 h in a Bouin solution consisting of picric, acetic acids and formalin (15:1:5), then the samples were embedded in paraffin to prepare histological sections of a 5-6 μm thickness [12, 13]. The preparations were stained with hematoxylin and eosin (BioVitrum, Russia).

During histological analysis, the seminiferous tubules having a rounded shape and a lumen (transverse section) were examined. Cell types of spermatogenic epithelium were identified morphologically [14, 15]. Histological preparations were examined using light microscopy (Ni-U, Nikon, Japan; the microscope is equipped with a software for image processing and analysis NIS-Elements, Nikon, Japan). The diameter of the seminiferous tubules, the number and types of spermatogenic cells located in them were evaluated.

Statistical processing was performed using the MS Excel 2016 data analysis package (t-test). The tables present arithmetic means (M) and standard errors of

means (±SEM). Differences were considered statistically significant at $p < 0.01$.

Results. The histological structure of the testes in quail was similar to that in mammals. The parenchymal tissue of the organ was formed by a system of convoluted seminiferous tubules containing various types of cells (Sertoli cells, spermatogonia, spermatocytes, spermatids, and sperm cells).

The size of the seminiferous tubules in quail varied during ontogenesis (Table 1). This indicator slightly increased with age during the early postnatal development. The diameter of the seminiferous tubules increased by 69% ($p < 0.01$) from week 1 to week 2, by 28% ($p < 0.01$) from week 2 to week 3, and by 29% ($p < 0.01$) from week 3 to week 4. From week 4 to week 5, an increase in the size of the seminiferous tubules was the most apparent. The diameter and area of the seminiferous tubules in 5-week-old quail were 2 times greater than that in individuals aged 4 weeks. From week 6 to week 24, the sizes of the seminiferous tubules almost did not change.

1. Age-related dynamics of morphological parameters of the testis histostructure in Estonian quail (*Coturnix coturnix*) ($n = 80$, M±SEM)

Age, weeks	Diameter of seminiferous tubules, microns	Area of seminiferous tubules, μm^2	Number of spermatogenic cells per seminiferous tubule
1	42±1	1329±55	18±1
2	71±2^{ab}	4117±222^{ab}	24±1^{ab}
3	91±2^{ab}	6172±177^{ab}	58±4^{ab}
4	117±2^{ab}	9932±310^{ab}	80±6^{ab}
5	237±4^{ab}	40688±993^{ab}	249±16^{ab}
6	278±5^{ab}	56232±868^{ab}	587±34^{ab}
12	282±7^{ac}	57023±766^{ac}	598±23^{ac}
24	291±6 ^{ac}	55985±812^{ac}	570±41^{ac}

$^{a, b}$ Differences with the previous age group are statistically significant at $p < 0.01$.

$^{a, c}$ Differences with the same indicator at the age of 1 week are statistically significant at $p < 0.01$.

An increase in the diameter and area of the seminiferous tubules in quail with aging was due to the growth and differentiation of spermatogenic cells (Table 1). In 1-week-old males, the number of spermatogenic cells in one seminiferous tubule ranged from 11 to 26 and averaged 18±1. In 2-week-old males, this indicator was 33% ($p < 0.01$) higher compared to 1-week-old animals, and it was 142% ($p < 0.01$) higher from week 2 to week 3, and 86% ($p < 0.01$) higher from week 3 to week 4. Significant growth and differentiation of spermatogenic cells were recorded from week 4 to week 6. In the seminiferous tubules of 5-week-old males, as compared to 4-week-old males, the number of spermatogenic cells increased 3.1-fold, from week 5 to week 6 2-fold. After reaching maturity (6 weeks), the growth and development of males practically were not accompanied by changes in the number of spermatogenic cells in the seminiferous tubules of the testes. The differences in this indicator, established in males aged 6 weeks and at a later age (3 and 6 months), did not exceed 1.8%.

The presence, number, and ratio of spermatogenic cells inside the seminiferous tubules of the testes varied depending on the age of the quail (Table 2). In 1-week-old quail, the basal membrane of the seminiferous tubules of the testes was lined with Sertoli cells and single spermatogonia (Fig. 1, A), while the number of Sertoli cells prevailed with a percentage of 71% vs. 29%, respectively. Sertoli cells had a dark-colored nucleus of a pyramidal shape located on the basal membrane. Spermatogonia were located along the seminiferous tubule but not on the basal membrane; they were mainly represented by type A (testis stem cells). Cells of this type were large and were characterized by the presence of a nucleus of an elliptical or round shape, located usually on the basal membrane of the tubule. Nuclear chromatin in the core was concentrated in one area.

2. Characterization of the spermatogenic epithelial cells population in the seminiferous tubules of the testes of Estonian quail (*Coturnix coturnix*) of different ages (n = 80, M±SEM)

Age, weeks	Cell type				
	Sertoli cells	spermatogonia	1st-order spermatocytes	2nd-order spermatocytes	spermatids
1	12±1	5±1	0	0	0
2	13±1	10±1[ab]	0	0	0
3	15±1	44±4[ab]	0	–	0
4	14±1	49±3	11±1	5±1	0
5	16±1	92±5[ab]	21±3[ab]	25±3[ab]	96±13
6	16±1	112±4[ab]	61±5[ab]	84±2[ab]	192±3[ab]
3	18±3	113±9	65±5	95±8	194±5
6	18±2	119±6	63±4	93±4	192±3
12	12±1	5±1	0	0	0
24	13±1	10±1[ab]	0	0	0

[a, b] Differences with the previous age group are statistically significant at $p < 0.01$.

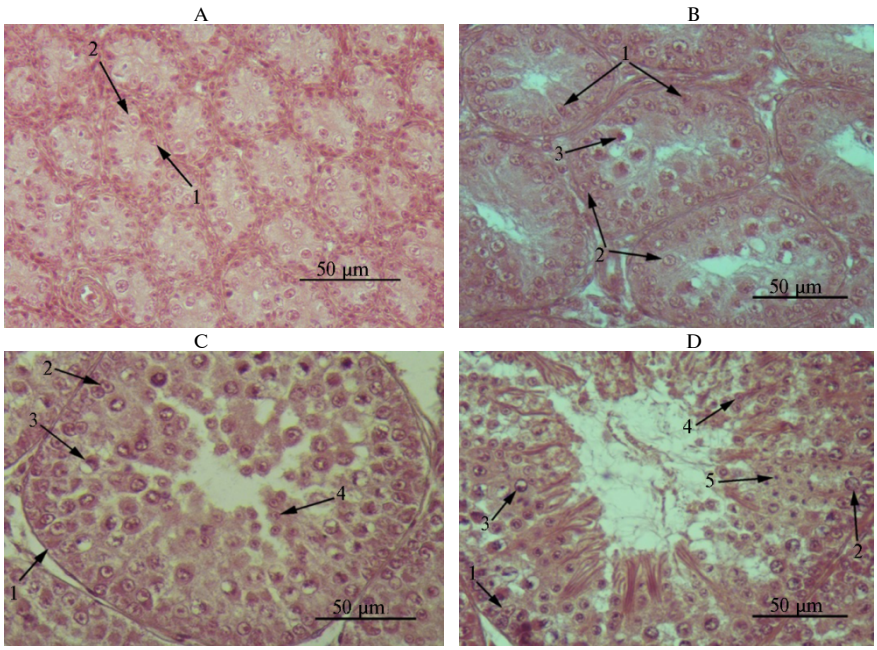

Fig. 1. Histological structure of the seminiferous tubules of the testes of Estonian quail (*Coturnix coturnix*) at the age of 1 week (A)**, 3 weeks** (B)**, 4 weeks** (C) **and 6 weeks** (D)**:** 1 — Sertoli cells, 2 — spermatogonia, 3 — 1st-order spermatocytes, 4 — 2nd-order spermatocytes, 5 — spermatids. Hematoxylin and eosin staining, light microscopy (Ni-U, Nikon, Japan), magnification ×400.

In 2-week-old quail, spermatogenic epithelial cells of the seminiferous tubules were also represented by two types — Sertoli cells and spermatogonia. The number of spermatogonia of various types in the seminiferous tubule increased to 10±1 ($p < 0.01$). At the same time, the number of Sertoli cells changed insignificantly (see Table 2). Spermatogonia were found both on the periphery and inside the seminiferous tubule. Along with type A spermatogonia, we detected spermatogonia of the intermediate type and type B. Spermatogonia of the intermediate type were slightly smaller than type A spermatogonia, the chromatin in their nucleus merged into one or two nucleoli and had a darker color. Type B spermatogonia were characterized by the presence of a large round or elliptical nucleus; chromatin flakes in the nucleus were distributed throughout the endoplasm. At this stage of development, the lumen in the seminiferous tubule was absent.

Development in the seminiferous tubules of the lumen necessary for the

release of sperm in adults was recorded at the age of 3 weeks (see Fig. 1, B). Spermatogonia were located on the basal membrane. Their number compared to the previous period increased 4.4 times ($p < 0.01$).

At the age of 4 weeks, small developing lumens were present in all seminiferous tubules. In some of them, spermatogenic epithelial cells were lined up in 5-6 rows. Along with Sertoli cells and spermatogonia, there were spermatocytes of the 1st and 2nd order, located closer to the center of the tubule. First-order spermatocytes were large cells with a large oval nucleus. Second-order spermatocytes were smaller in size (see Fig. 1, B). The average number of 1st- and 2nd-order spermatocytes in the seminiferous tubule did not exceed 11±1 and 5±1, respectively. The number of Sertoli cells and spermatogonia at this age did not noticeably change compared to the previous period: differences for indicators did not exceed 6 and 13%, respectively.

In 5-week-old animals, the population of spermatogenic cells in the seminiferous tubules was represented by Sertoli cells, spermatogonia located on the basal membrane, and spermatocytes of the 1st and 2nd order (see Table 2). Near the lumen, the presence of spermatids in the form of immature small formations of a rounded shape with a clearly visible nucleus was recorded. Individual spermatozoa occurred in the lumen of some seminiferous tubules.

At the age of 6 weeks, elongated spermatids with tails occurred near the lumen of the seminiferous tubule; their number increased 2.0-fold compared to the previous period ($p < 0.01$, see Table 2, Fig. 1, D). The presence of spermatids at different stages of spermiogenesis was recorded, which led to their unequal shape.

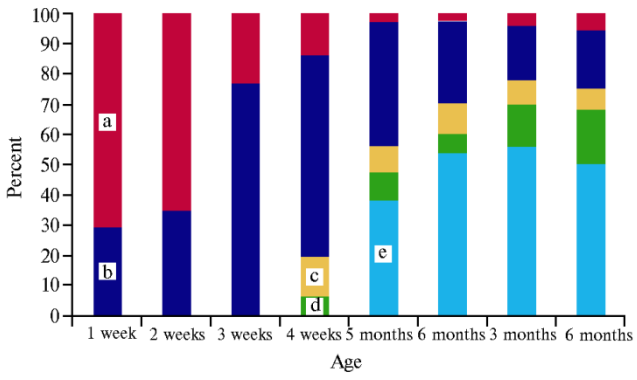

Fig. 2. The ratio of spermatogenic epithelial cells of the seminiferous tubules in the testes of Estonian quail (*Coturnix coturnix*) of different ages: a — Sertoli cells, b — spermatogonia, c — 1st-order spermatocytes, d — 2nd-order spermatocytes, e — spermatids.

So, with approaching the lumen of the seminiferous tubules, spermatids decreased in size and were cells with an oval elongated head of dark color and long tails. In individual seminiferous tubules, spermatids in the form of bundles were found. Developed spermatozoa were in the lumens of most seminiferous tubules. Sertoli cells often had an elongated shape (in the form of strands located almost from the basal membrane to the lumen of the tubule). The total number of cells in the seminiferous tubules compared to the 5 weeks of age was higher 2.4 times mainly due to an increase in the number of spermatocytes, spermatids, and spermatozoa ($p < 0.01$).

In adult males aged 3 and 6 months, all spermatogenic epithelial cells were present in the seminiferous tubules; the average values of these indicators were almost the same. Differences in the content in the seminiferous tubules of spermatogenic cells of different types, found in 6-week-old and 3- and 6 month-old quail, did not exceed 11%.

Thus, the studies showed that in quail of different ages, the number of different types of spermatogenic cells and their ratio in the seminiferous tubules of the testes vary. The share of Sertoli cells in the total number of spermatogenic cells in the seminiferous tubule decreased with age due to spermatocytes and

spermatids (Fig. 2). During the early period of postnatal development of males, the proportion of spermatogonia increased as the animal matured, reaching a maximum value (up to 76.6%) by the age of 3 weeks. In the subsequent period, this indicator decreased to 13% at the age of 6 months.

The results of this study are consistent with the data that the authors obtained earlier in studying the age-related characteristics of spermatogenesis in rabbits [19], roosters [20], and guinea fowls [21]. In males of these species, the composition of spermatogenic cells in the seminiferous tubules of the testes is shown to change during ontogenesis. During the postnatal period, an uneven increase in the size of the seminiferous tubules and differences in the presence, number and ratio of spermatogenic cells in them occur. During the early ontogenesis, the size of the seminiferous tubules and the number of spermatogenic cells in them constantly and significantly increase. Intensive growth and differentiation of spermatogenic cells and, as a result, an increase in the size of the seminiferous tubules are detected in the period preceding the maturity: in male rabbits between the ages of 5 and 6 months, in roosters and guinea fowls from 4 to 5 months. It should be noted that similar studies performed by other authors were not found.

Among the available information sources, we also did not find papers on age-related changes in the morphometric characteristics of the seminiferous tubules of the testes in quail and the quantitative composition of spermatogenic cells. Studies on the birds, including quail, were mainly associated with the morphometric indicators (diameter, area, etc.) of spermatogenic cells and their structural units in the course of differentiation [22, 23]. There are a number of papers of other authors on the study of the anatomical structure and morphometric data of the genitals in male quail [24-27]. So, the morphometric parameters of the testes were assessed for 60-day-old quail, in particular, the sizes of the testes, their anatomical structure and histological structure [24]. The results of histological studies presented in this paper are consistent with those we obtained. Kannan et al. [25] studied the anatomical and morphological features of quail testes in age dynamics, in young quail, upon reaching maturity and in adult males. An increase in the size of the testes to the age of 22 weeks has been shown. Similar data were obtained by Bausova [26]. A number of studies have noted the effect of various feeds, hormones, herbicides and other substances [28-30], as well as natural and artificial lighting, on quail spermatogenesis [31].

So, in quail (*Coturnix coturnix*), the size of the seminiferous tubules, the number and composition of spermatogenic cells in them varies depending on age. At the age of 1-3 weeks, the population of cells of the epithelial spermatogenic layer of the seminiferous tubules is represented by two types — Sertoli cells and spermatogonia. In 3-week-old animals, the seminal lumen begins to develop, the histological sections of the testes show spermatocytes of the 1st and 2nd order from week 4, and spermatids from week 5. In the seminiferous tubules of the 6-week quail, all types of cells of the epithelial spermatogenic layer are present, i.e. Sertoli cells, spermatogonia of various types, spermatocytes of the 1st and 2nd order, spermatids, and sperm cells. These data expand the understanding of the morphology of developing germ cells in male poultry and suggest that quail age from 1 to 3 weeks is the optimal period for manipulating spermatogonia when used as biological material to preserve the genetic resources of poultry in cryobanks, as well as targets for the introduction of gene constructs to obtain transgenic birds.

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