

## AGE DYNAMICS OF SPERMATOGENESIS IN COCKS IN CONNECTION WITH OPTIMIZATION OF BIOENGINEERING MANIPULATION TIME

E.V. Beloglazova<sup>1</sup>, T.O. Kotova<sup>1</sup>, N.A. Volkova<sup>1</sup>, L.A. Volkova<sup>1</sup>,  
N.A. Zinovieva<sup>1</sup>, L.K. Ernst<sup>2</sup><sup>1</sup>All-Russia Research and Development Institute for Livestock Husbandry, RAAS,  
Moscow province, Podolsk region, Dubrovitsy 142132, Russia

e-mail: natavolkova@inbox.ru

<sup>2</sup>Russian Academy of Agricultural Sciences, Moscow 117218, Russia

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## Summary

In multiple age cocks at the age of 1 week and 12 months the authors studied the morphological structure of testicle tissue and distribution of spermatogenic epithelium cells in seminal tubules. It was established, that the optimal age for bioengineering manipulations with sexual cells of cocks testicles is period from 1 to 6 weeks, when generative cells of seminal tubules correspond substantially to the spermatogonium.

**Keywords:** cock, testis, spermatogonia, spermatogenesis.

Using reproductive cells is considered as one of the promising methods for creation transgenic animals including bird (1-3). There are different available techniques on transfer of genetic information in embryonic cells of the bird (4-9). Is of a particular interest the use of testicle cells owing to their natural ability to absorb foreign DNA (fDNA) and bring it into the egg during fertilization. At the same time, fDNA integrated into the genome can be stably transmitted over generations. Bioengineering manipulations on adult animals significantly reduce costs of material and time on obtaining transgenic progeny.

Spermatogonia are the cells of spermatogenic epithelium most interesting for researchers. A population of these cells is constantly being renewed, which provides great opportunities to realize their potential when creating transgenic birds. DNA replication in spermatogonia initiates a complex process of their differentiation resulting in highly specialized reproductive cells - spermatozoa.

The purpose of this work was to study the dynamics of spermatogenesis in cocks and determine the optimal time for introducing recombinant DNA in male reproductive cells at early stages of differentiation in order to improve the procedures on obtaining transgenic poultry.

*Technique.* Histological study of the testis tissue of cocks ( $n = 65$ ) was performed in 19 age categories (from 1 week to 12 months, each group of 5 individuals), the material was collected at slaughter of the bird.

The testes were fixed with Bouin's solution (15 ml picric acid, 5 ml 40% formalin, 1 ml glacial acetic acid) for at least 24 h. Dehydration and paraffin embedding of tissue samples were carried out using conventional techniques (10). Tissue sections were prepared on a rotary microtome, stained with hematoxylin and eosin solutions ("BioVitrum", Russia) and examined using a microscope by "Opton" (Germany) (lenses  $\times 40$ ,  $\times 16$ ) and the program Image Scope ("Systems for Microscopy and Analysis", Moscow). Only the round-shaped seminal tubules were accounted. At least 30 seminal tubules were investigated in each individual. Cell morphology of spermatogenic epithelium was identified.

Statistical processing of data was performed in Microsoft Excel.

*Results.* Histostructure of the cocks' testes was found to be similar to that of mammals. Parenchyma included multiple twisted seminiferous tubules merging in mediastinum in straight tubules which ran into the efferent tubules. Each twisted seminal tubule was covered with connective tissue whose inner layer was the basal membrane carrying spermatogenic epithelium. This layer was represented by two populations of cells - sustentacular cells (Sertoli cells) and spermatogenic cells at different stages of differentiation (spermatogonia, spermatocytes, spermatids, spermatozoa).

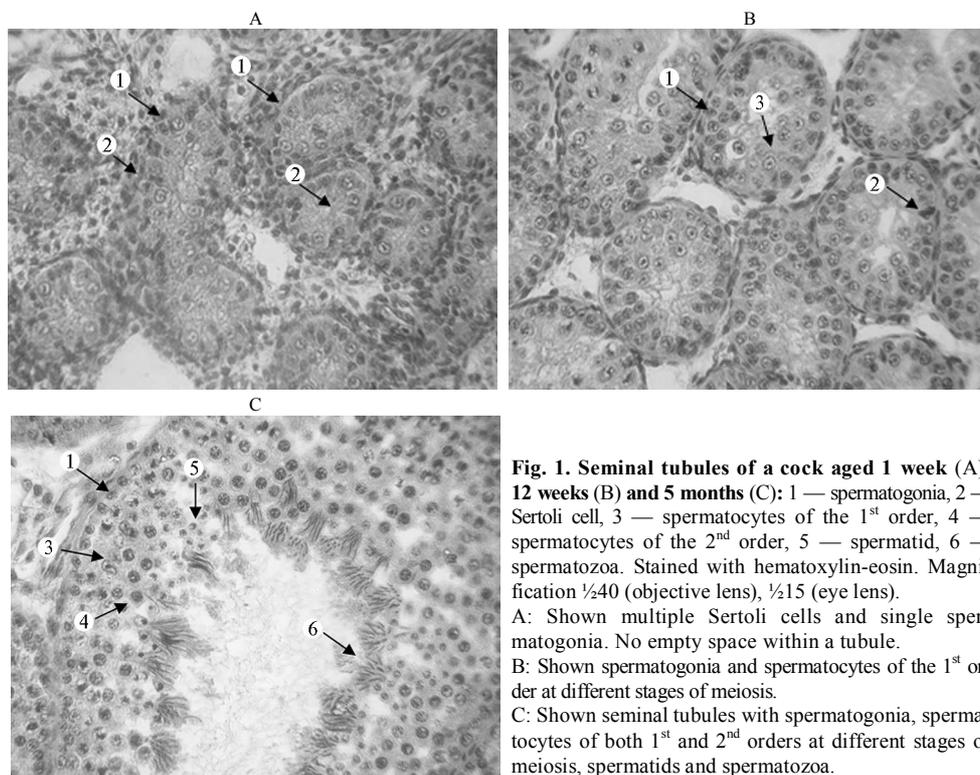
At older age, the number of seminal tubules per unit area of testis tissue changed (Table). In the age period up to 12 weeks, the changes were minor: the number of tubules per 1 mm<sup>2</sup> section reduced by an average 46,3% (from 201  $\pm$  22 to 108  $\pm$  12 pcs.) at a 2,5-fold increase in number of cells in the tubule (from 25,0  $\pm$  0,3 to 62,0  $\pm$  0,4 pcs.). Later (from 3 to 6 months), the changes became more expressed. Tubules became much larger while the average number of tubules per unit area of a section reduced (by 18  $\pm$  2 pcs. per 1 mm<sup>2</sup>, or 6 times as compared with the previous period). This fact occurred primarily due to the increase in number of cells of the spermatogenic epithelial layer of seminal tubules (up to 1153,9  $\pm$  7,6 pcs., or over 18 times than that at the age of 3 months). At the period from 6 to 12 month, there were almost no changes in size and number of cells in the tubules.

**Age dynamics of morphological characteristics ( $X \pm x$ ) of histological structure of cocks' testicles**

| Age      | Mean, pcs.                          |  | Diameter of seminal tubules, $\mu\text{m}$ | Area of seminal tubules, $\mu\text{m}^2$ |
|----------|-------------------------------------|--|--|--|
|          | seminal tubules per 1 $\text{mm}^2$ | spermatogenic cells per a seminal tubule |  |  |
| 1 week   | 201 $\pm$ 22                        | 25,0 $\pm$ 0,3                           | 50,5 $\pm$ 1,2                             | 1871 $\pm$ 82                            |
| 2 weeks  | 188 $\pm$ 9                         | 30,0 $\pm$ 0,3                           | 61,4 $\pm$ 1,1                             | 2715 $\pm$ 93                            |
| 3 weeks  | 170 $\pm$ 7                         | 30,0 $\pm$ 0,2                           | 60,8 $\pm$ 0,9                             | 2638 $\pm$ 74                            |
| 4 weeks  | 145 $\pm$ 9                         | 31,0 $\pm$ 0,3                           | 62,1 $\pm$ 0,5                             | 2771 $\pm$ 40                            |
| 5 weeks  | 136 $\pm$ 8                         | 30,0 $\pm$ 0,3                           | 62,1 $\pm$ 0,6                             | 2795 $\pm$ 59                            |
| 6 weeks  | 127 $\pm$ 6                         | 40,0 $\pm$ 0,4                           | 67,0 $\pm$ 0,8                             | 3207 $\pm$ 71                            |
| 7 weeks  | 129 $\pm$ 11                        | 38,0 $\pm$ 0,4                           | 66,7 $\pm$ 0,6                             | 3203 $\pm$ 55                            |
| 8 weeks  | 131 $\pm$ 9                         | 44,0 $\pm$ 0,4                           | 65,0 $\pm$ 0,8                             | 3036 $\pm$ 76                            |
| 9 weeks  | 122 $\pm$ 10                        | 50,0 $\pm$ 0,8                           | 68,0 $\pm$ 0,9                             | 3331 $\pm$ 97                            |
| 10 weeks | 116 $\pm$ 9                         | 58,0 $\pm$ 0,5                           | 69,1 $\pm$ 1,0                             | 3368 $\pm$ 90                            |

|            |        |             |            |            |
|------------|--------|-------------|------------|------------|
| 11 weeks   | 117±8  | 54,0±0,5    | 73,0±1,5   | 3862±153   |
| 12 weeks   | 108±12 | 62,0±0,4    | 86,2±0,9   | 5348±116   |
| 3,5 months | 88±3   | 70,0±0,6    | 97,2±2,3   | 6963±306   |
| 4 months   | 74±7   | 226,0±1,7   | 111,7±1,1  | 8962±172   |
| 4,5 months | 62±6   | 291,0±1,8   | 169,0±10,8 | 27787±282  |
| 5 months   | 17±2   | 711,0±5,6   | 191,9±2,0  | 27092±564  |
| 5,5 months | 15±2   | 913,0±6,0   | 195,0±2,6  | 27877±525  |
| 6 months   | 18±2   | 1154,0±7,6  | 186,8±2,0  | 23760±491  |
| 12 months  | 15±3   | 1148,0±10,6 | 216,2±6,3  | 36254±2297 |

The age-related changes in histostructure of cocks' testicular tissue reflected the development of cells in spermatogenic epithelium. In testicles of cocks aged 7 days, seminal tubules contained only two types of cells: Sertoli cells and young generative cells – spermatogonia (respectively  $16,0 \pm 0,3$  and  $9,0 \pm 0,2$  pcs.). (Fig. 1, A). Sertoli cells were located peripherally; the nucleus of these cells – pyramidal or oval, located perpendicular to the basal membrane. Spermatogonia contained a pale cytoplasm and large nucleus located both at the periphery and within seminal tubules. At this stage, seminal tubules had no internal space.



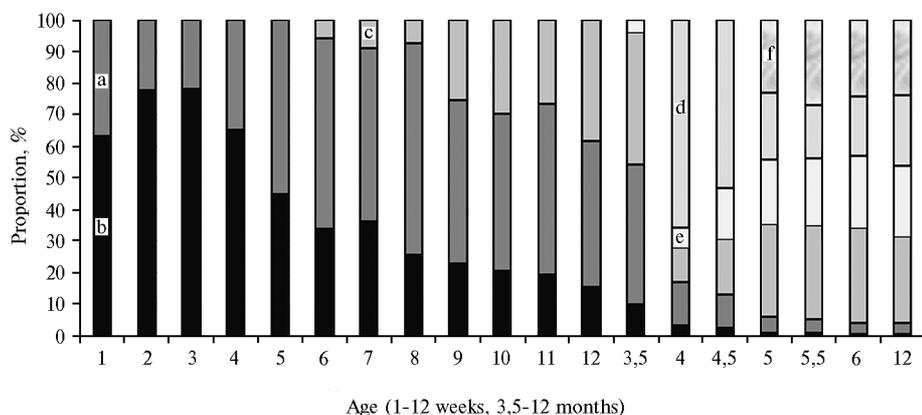
**Fig. 1. Seminal tubules of a cock aged 1 week (A), 12 weeks (B) and 5 months (C):** 1 — spermatogonia, 2 — Sertoli cell, 3 — spermatocytes of the 1<sup>st</sup> order, 4 — spermatocytes of the 2<sup>nd</sup> order, 5 — spermatid, 6 — spermatozoa. Stained with hematoxylin-eosin. Magnification  $\frac{1}{2}40$  (objective lens),  $\frac{1}{2}15$  (eye lens).  
A: Shown multiple Sertoli cells and single spermatogonia. No empty space within a tubule.  
B: Shown spermatogonia and spermatocytes of the 1<sup>st</sup> order at different stages of meiosis.  
C: Shown seminal tubules with spermatogonia, spermatocytes of both 1<sup>st</sup> and 2<sup>nd</sup> orders at different stages of meiosis, spermatids and spermatozoa.

At the age of 14 days, spermatogonia were located at the periphery of seminal tubules. Interstitial cells were surrounded by loose connective tissue between loops of seminal tubules. The number of Sertoli cells in tubules increased to  $23,0 \pm 0,2$  pcs., the number of spermatogonia didn't change -  $7,0 \pm 0,1$  units. At the age of 3 weeks, a space appeared in the center of seminal tubules while no changes in number of generative cells. In 4-week-old cocks, the number of Sertoli cells in seminal tubules decreased to  $20,0 \pm 0,2$  pcs., a number of spermatogonia slightly increased (up to  $11,0 \pm 0,1$  pcs.).

At the age of 5 weeks, a space was detected in almost all tubules. Spermatogenic cells were located on basal membrane. A physiological loss of Sertoli cells was observed: their number reduced to  $14,0 \pm 0,3$ . On the contrary, the number of spermatogonia increased up to  $15,5 \pm 0,2$  pcs. Intermediate cells – spermatogonia at stages of prophase and metaphase - were observed. In cocks aged 6-7 weeks, the size of seminal tubules continued to grow as well as the size of internal space. Spermatogonia were located as almost continuous layer between Sertoli cells. The number of Sertoli cells was almost the same ( $13,0 \pm 0,2$  pcs.), spermatozoa - increased to  $24,1 \pm 0,2$  pcs. A new type of spermatogenic cells appeared - spermatocytes of the 1<sup>st</sup> order ( $3,3 \pm 0,2$  pcs.). These cells were detected in the second row on membrane; they had a round shape, large nucleus and light cytoplasm.

At the age of 8-12 weeks, there occurred a slight increase in number of cells of spermatogenic epithelium (Fig. 1, B). Sertoli cells were located on the wall of tubules in a row, and between them there was the almost continuous layer of spermatogonia, whose number remained almost the same ( $26,0 \pm 0,1$  to  $29,0 \pm 0,2$  pcs.). The number of spermatocytes of the 1<sup>st</sup> order increased up to  $24,0 \pm 0,2$  pcs. In seminal tubules of cocks aged 3,5 months, spermatocytes of the 2<sup>nd</sup> order were detected (less than  $2,7 \pm 0,1$  pcs.). In cocks aged 4 months, seminal tubules contained almost all types of reproductive cells except spermatozoa. A continuous layer of spermatogonia was located on the basal membrane, between them – Sertoli cells of a typical pyramidal shape. Above spermatogonia, there were 2-3 layers of spermatocytes and young spermatids. The number of Sertoli cells amounted to  $7,0 \pm 0,1$ , spermatogonia -  $31,0 \pm 0,7$ , primary and secondary spermatocytes at various stages of meiosis - respectively  $25,0 \pm 0,4$  and  $14,5 \pm 0,4$ , young round-shaped spermatids -  $148,0 \pm 2,7$  pcs.

At the age of 4,5-5 months, spermatogenic cells reached the level of pyriform spermatids and mature spermatozoa (Fig. 1, B). All types of spermatogenic cells peculiar to active spermatogenesis were observed. Most of seminal tubules contained mature spermatozoa. At this age, the number of cells varied within the following limits: spermatogonia -  $36,0 \pm 2,0$ ; spermatocytes of the 1<sup>st</sup> and 2<sup>nd</sup> orders -, respectively  $209,0 \pm 4,7$  and  $146,0 \pm 1,8$ ; spermatids -  $151,0 \pm 1,9$ ; spermatozoa -  $162,0 \pm 2,1$  and Sertoli cells -  $7,0 \pm 0,1$  pcs. In cocks aged 6 and 12 months, the number of cells was almost unchanged: spermatogonia – respectively  $40 \pm 2,5$  and  $38,0 \pm 1,4$ ; spermatocytes of the 1<sup>st</sup> order -  $350,0 \pm 1,4$  and  $321,0 \pm 7,4$ , spermatocytes of the 2<sup>nd</sup> order -  $263,0 \pm 5,8$  and  $262,0 \pm 3,8$ ; spermatids -  $216,0 \pm 4,2$  and  $262,0 \pm 3,8$ ; spermatozoa -  $279,0 \pm 1,6$  and  $277,0 \pm 3,6$ ; Sertoli cells –  $6,0 \pm 0,2$  and  $7,0 \pm 0,3$  pcs.



**Fig. 2. Age dynamics in morphological composition of spermatogenic epithelium of seminal tubules in cocks' testicles:** a — spermatogonia, b — Sertoli cells, c — spermatocytes of the 1<sup>st</sup> order, d — spermatids, e — spermatocytes of the 2<sup>nd</sup> order, f — spermatozoa.

Therefore, in cocks aged 1-6 weeks spermatogonia were the main type of cells in spermatogenic epithelium of seminal tubules (Fig. 2). The maximum number of these cells was detected in testes of cocks aged 5 months. At the age of 6 weeks in seminal tubules appeared spermatocytes of the 1<sup>st</sup> order, and from 3,5-4 months – spermatogonia of the 2<sup>nd</sup> order. Spermatozoa were found in testicular tissue of cocks aged 5 months; the number these cells increased up to the 6-months-age and then remained almost unchanged until the age of 12 months.

So, the optimal age for bioengineering manipulations on male reproductive cells of cocks is 1-6 weeks, because spermatogonia are the main type of cells in seminal tubules of cocks' testes at this period.

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