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THE APPLICATION OF BUSULFAN TO INHIBIT THE SPERMATOGENESIS IN QUAIL TESTIS

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Introduction

The use of testicular stem cells (spermatogonia) is of most interest for obtaining individuals with predetermined traits and genome genetic modification and for conservation of poultry gene pool. A significant population of mature donor germ cells (sperm) is formed upon successful spermatogonia cells transplantation into the testes of male recipients. Obtained sperm can be used to produce offspring with the desired traits. A key step in this technology is the removal of own spermatogenic cells (inhibition of spermatogenesis) in male recipients.

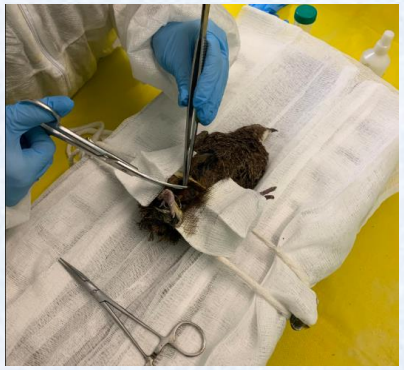
The aim of research was to develop and optimize methodological approaches to inhibit the spermatogenesis in quail using busulfan.

Material and methods

The busulfan drug was injected directly into the testes parenchyma of mature males by multiple injection at the concentration from 20 to 100 mg per 1kg of body weight (n=25). Histological preparations of testes from the experimental quails were obtained to study composition of spermatogenic cells in the seminiferous tubules after busulfan administration.

The male peers who were not injected with busulfan were used as a control.

the males of quail



Operation for the busulfan introduction in the testes of quail

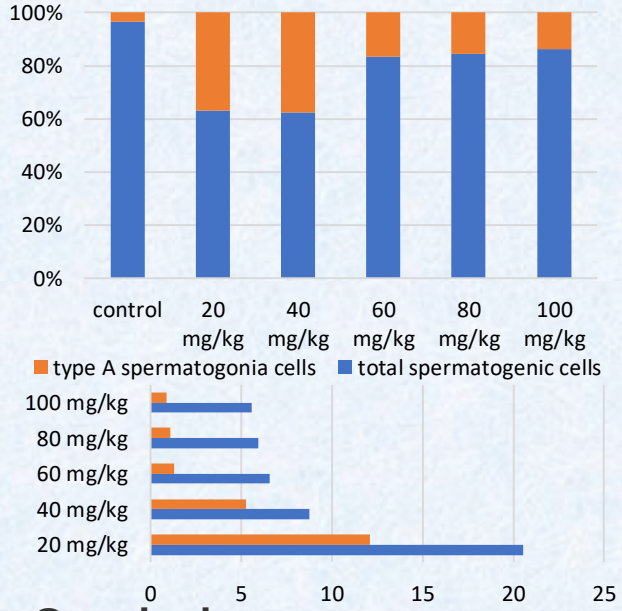
Results

Experimental quails showed a decrease in the number of spermatogenic cells in the seminiferous tubules 32, 75, 111, 119 and 118 times compared with the control when using busulfan in concentrations 20, 40, 60, 80 and 100 mg/kg of weight, respectively ($p < 0.001$).

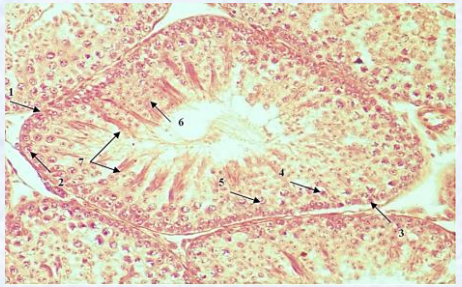
The cells composition in the seminiferous tubules from experimental quails was represented mainly by Sertoli cells and type A spermatogonia. After busulfan introduction at the concentrations 20, 40, 60, 80 and 100 mg/kg, the percentage of spermatogonia was 55±5 %, 24±4 %, 6±2 %, 5±2 % and 4±1 %, respectively.

The use of busulfan at the concentration of 80-100 mg/kg led to high mortality of quails.

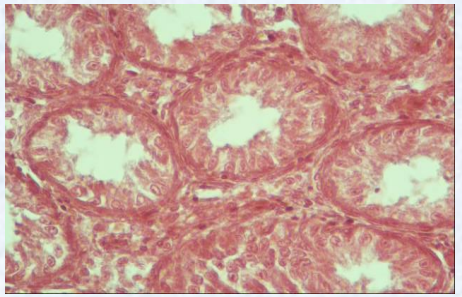
The percentage of type A spermatogonia cells in the tubules



Cross section of mature quails seminiferous tubules



Increase x20
1 - type A spermatogonia,
2 - intermediate spermatogonia,
3 - type B spermatogonia,
4 – primary spermatocyte,
5 - secondary spermatocyte,
6 - spermatid,
7 - spermatozoon.



No germ cells in the seminiferous tubules after 80 mg/kg busulfan treatment

Conclusion: it was found that the optimal busulfan concentration for elimination of quail spermatogenic cells was 60 mg/kg.

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