SLOVENSKÁ POĽNOHOSPODÁRSKA UNIVERZITA V NITRE
FAKULTA BIOTECHNOLÓGIE
A POTRAVINÁRSTVA

Pracovisko / Katedra biochémie a biotechnológie

Reviewer´s comments on PhD thesis

The author of the PhD thesis: Mgr Inż. Michalina Debowska
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The title of PhD thesis:
Sperm and primordial germ cells as a model for interpretation of competition test results

Supervisor: prof. Dr. Hab. Marek Bednarczyk,
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Reviewer: prof. Ing. Peter Chrenek, DrSc.
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The doctoral dissertation generally regards to the importance of biodiversity, especially to conservation methods using chicken sperm competition and primordial germ cells (PGCs), which are precursors for oocytes and sperm cells. The genetic diversity of the characteristics that are of great interest to animal breeders is the basis for future breeding program and animal genetic resource protection. Variability is represented by genetic differences between individuals, families and populations within a species. New molecular technology provides new opportunities for assessing genetic variability at the DNA level.

Moreover, genetic distance studies using microsatellite markers have been performed in many animal species including chicken also. These methods do not provide an information about successful *in vitro* or *in vivo* fertilisation. One of the best test can be sperm competition, since the genetic distance between the sperm donor and the sperm recipients appears to be one of the factors that may affect the results of artificial fertilization in chicken. Sperm competition can be an important factor of sexual selection. There are several mechanisms that have allowed the adaptation of the bird reproductive system to the phenomenon of sperm competition.

In this PhD work author also determines if there is a connection between the bird´s genetic distance and results of the sperm competition test, and whether similar results would be obtained on sperm cells precursors, which are primordial germ cells.

Genetic distance research has been used for many years as a technique to support the cryopreservation of animal biodiversity.

The thesis meets all requirements for such type of documents. It is clearly written and properly divided into 7 chapters. In the literature review, written on 10 pages, PhD student analyses relationship between the genetic similarity of birds and the results of semen and primordial germ cells competition test. Totally 91 domestic and foreign literary sources were used in this paper, however, most of cited sources are originated from older years.

To achieve all the goals, such as DNA isolation, sperm evaluation, PGC isolation, and allele detection in populations, appropriate methods have been proposed. The task is clear and logical and characterizes the bird biodiversity in detail as well as describes the characterization of sperm and PGC. Formally, the study is well-written, but contains several mistakes or misprints. Firstly, the title of PhD thesis should be corrected for "Sperm and primordial germ cells as a model for interpretation of competition test results ". The goals are designed briefly and clear.

The chapter „Material and Methods" describes three research methodologies, particularly, determination the genetic distance between populations, sperm competition test and primordial germ cell competition test. The experiments were focused on allele detection

also using microsatellite markers, genetic variability of the chicken sperm and primordial germ cell isolation from the gonads of 6-day old chicken embryos. Statistical parameters, characteristic for microsatellite marker analysis, were also determined using different methods. Methodical approaches chosen for this study were adequate to the aims of the study.

Obtained results and the novelty of the dissertation

PhD thesis is focused on the actual research area to determine the relationship between the genetic similarity of birds and the results of semen and primordial germ cells competition test. PhD student presents obtained results in an appropriate form using tables, charts and figures. Various breed types and sufficient amount of alleles in this study were used to determine sperm and PGCs competitions and genetic distance. The student had a lot of data that were reasonably evaluated, supplemented by tables and charts, which help to better understand the problematics, what is sufficient for this type of work.

The effect of genetic distance on the results of sperm competition has been demonstrated. The effect of genetic distance on the results of primordial germ cell competition was also demonstrated. The results are applicable in the biotechnology, agriculture field as well as in molecular biology.

The results of the experiments are presented clearly, what gives a possibility to use the techniques examined in this study for further manipulations with avian PGC.

Comments

In case of sperm quality, it is important to obtain the progressive movement parameters before AI (for example CASA analysis). Some environmental changes can influence the sperm motility and progressive movement parameters. The progressive movement is better indicator for AI than just total motility, therefore I recommend analysing progressive movement also. Please, use the uniform name for sperm (either sperm or spermatozoa).

Isolation of PGCs was done from 5.5- and 6-day old embryos. You should state uniformly the day of PGCs isolation due to the differences in PGCs numbers.

All the time you worked with PGCs, but in the methods there is no description how did you separate the PGCs from gonads (filter, one-by-one cell suction ...)?

There is missing information on how you isolated the blastoderm cells, how many cells did you use for analysis; some characteristics of blastodermal cells are required. Also add the chapter "statistical analyses".

Questions

1. It is known, that sperm concentration in birds (rooster) is higher than in mammals. Which extender did you use for semen dilution?
2. At which season did you obtain rooster semen? (spring, summer,…?)
3. How many PGCs did you obtain from one embryo using mechanical fragmentation of gonads and incubation for 1 hour in PBS?
5. Can these results be applied to other bird species?

Conclusion:

Presenting the above opinion to the Board of the Faculty of Animal Breeding and Biology at the UTP University of Science and Technology in Bydgoszcz, I confirm that evaluated PhD thesis „ **Sperm and primordial germ cells as a model for interpretation of competition test results**" corresponds fully to the defined requirements, specified by the "art. 26 ustawy z dnia 14.03.2003 r. o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki, z późniejszymi zmianami i Ustawą „Prawo o szkolnictwie wyższym".
On this basis, I recommend the admission of **Mgr Inż. Michalina Debowska** to the next stage of a PhD proceeding.

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At the same time, in the recognition of the enormous work done during the objectives pursued, using a wide panel of interdisciplinary techniques and taking into account the nature and significance of the results for the development of our knowledge about the behavior of primordial germ cells, I put the request to award the prize to the PhD thesis.

In Nitra, February, 7th, 2018

prof. Ing. Peter Chrenek, DrSc.