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Prof. dr hab. Dorota Zięba-Przybylska
Department of Animal Biotechnology
Faculty of Animal Science
Agricultural University in Krakow
31-120 Kraków
Al. Mickiewicza 21

Review of doctoral dissertation entitled:
"Maternal Immune Activation and Its Consequences on Offspring Behavior in an Ovine Model of Prenatal Infection"
written by Wiesława Florek, MSc

General description of the doctoral dissertation

The doctoral dissertation, submitted for evaluation, has been prepared at the Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences in Jastrzębiec under the supervision of dr hab. Anna Piliszek, prof. dr hab. Jacek Modliński and Dr Silvestre Sampino as Auxiliary Supervisor.

The dissertation, written in English, includes 130 pages and consists of ten chapters: *1) Abstract, 2) Polish Abstract, 3) Introduction, 4) Hypotheses, 5) Aims of the study, 6) Materials and Methods, 7) Results, 8) Discussion, 9) Conclusions, and 10) Bibliography.* In addition, the list of frequently used abbreviations has been included.

The analysis of the doctoral dissertation

The studies undertaken by Wiesława Florek focus on how maternal infection during pregnancy, which elicits *maternal immune activation* (MIA), has been associated with a higher risk of developing neurodevelopmental disorders in the offspring. In the dissertation, the Author primarily proves the effectiveness of using sheep as a research model for MIA in recalling several specific behavioral symptoms of humans.

The PhD dissertation discusses a critical topic I have been dealing with for over twenty years: using the sheep model to study human physiological processes. My research focuses on leptin resistance as a cause of obesity in humans. I believe that other animal models besides rodents are essential and are used in research on obesity or nervous diseases. But I know how difficult it is to convince others to use alternate animal models in research. With advances in obesity research, various animal models (e.g., mice, rats, pigs, and sheep) have been developed to investigate obesity pathogenesis, development, therapies, and complications. The choice of an obese model is based on factors including genetics, size, life expectancy, and generation time.

However, in many cases, financial constraints and the available animal care facilities limit the choices and have resulted in the wide usage of rodent models rather than large animals. A range of other species have unique attributes that allow different lines of investigation from those achievable with small laboratory rodents. In particular, domesticated animal species are amenable to specific impossible experimental procedures in smaller animals. An excellent example of this is the methodology for sampling CSF from III V in a conscious state, which was well-established as a technique 30 years ago and is the method of choice for measuring the output of hypothalamic hormones. The advantages of using livestock for neural, physiologic, and metabolic function studies are that larger animals allow serial sampling of blood, other bodily fluids, and tissues. Domesticated animals are not nocturnal, as rodents are, and critical daily patterns of behavior and physiology in the former group are more similar to those of humans. Environmental effects on behavior, food intake, and energy expenditure can be studied, and the role of factors such as the photoperiod has been well described, particularly in sheep. Mice are the species of choice for gene knockout and knockin studies. Sheep, which have strong seasonal breeding features, are characterized by circannual rhythms of the secretion of many hormones, food intake, and body weight, which makes them an elegant animal example for determining the neuroendocrine adaptations of animals to challenging environmental conditions. In the broadest sense, the behavioral model applicable to laboratory rats, mice, and humans also applies to larger animals, such as sheep, of similar body weight to humans.

Considering the advantages of domesticated large animals, the Author fills a gap in research by promoting sheep as a good model for human behavior research.

In the Introduction (total of 18 pages), the Author briefly presents the background of neurodevelopmental disorders and provides information about Autism Spectrum Disorder (ASD). Then, in 5 pages, she discusses an animal model of neurodevelopmental disorders, highlighting the advantages and disadvantages of individual models, focusing primarily on the rodent model. Another topic covered in the Introduction is MIA, paying attention to which inflammatory factors are activated during infection. The Introduction ends with characteristics of the alternate animal model of MIA, considering the possibility of using sheep as a model for *maternal immune activation* research.

In the following sections of the PhD thesis, the Author demonstrates the study's hypotheses and then presents the study's aims. The central hypothesis is that LPS administration during sheep pregnancy leads to *maternal immune activation* in pregnant ewes and elicits the development of behavioral aberrations in the resulting lamb, which resemble symptoms of neurodevelopment disorders. To answer whether the hypothesis is true, the Author set three research aims: 1. To investigate the effects of LPS-induced MIA during sheep pregnancy on lamb postnatal behavior; 2. To examine the inflammatory responses of the ewe to endotoxin-LPS administration, and 3. To investigate whether the intensity of the LPS-induced maternal inflammatory response during pregnancy may correlate with the severity of behavioral alternations in the lamb.

In the following chapter, Materials and Methods (22 pages), the Author introduces the reader to the description of the animal model she chose for research and experimental design. The experiments were carried out in three consecutive breeding seasons since the fall of 2016. She used a total of 90 primiparous Świniarka ewes, 30 per season, weighing 35 kg. During the breeding season, ewes were mated with the ram, and the pregnancy was identified by USG 40-

70 days post-coitum (pc). The Second Local Warsaw Ethics Committee procedures for Animal Experimentation approved all experimental procedures (no. WAW2/35/2017). Out of a group of 90 ewes, there were 85 pregnant ewes, and in spring, 76 lambs were born, including 44 females and 32 males; 10 lambs were excluded from the experiment due to miscarriage, post-partum rejection, stillborn lambs, and twins. To activate the immune system in pregnant ewes, LPS was intravenously administered in 2 ml solution – LPS-treated sheep and 2 ml of saline – to Control ewes. The treatments were scheduled for sheep: Experiment **1**.: MIA animals: MIA 125 group – LPS was injected at 125 dpc in a 1.2 µg/kg b.w.; Experiment **2**: MIA 85 group – LPS was injected at 85 dpc in a 1.2 µg/kg b.w.; and Experiment **3**: MIA 85' group - LPS was injected three times at 85, 86, and 97 dpc in doses approximately of 0.4 µg/kg b.w., 0.8 µg/kg b.w. and 1.2 µg/kg b.w. Ewe-lamb dyads were monitored after delivery and were subjected to different behavioral tests: isolation test to evaluate lambs' social attachment to their mother, V-detour test to assess inhibitory control towards a visual stimulus, as well as to measure spatial learning, memory, and different aspects of cognition, and T-maze test to evaluate spatial learning and cognitive flexibility under the various experimental paradigm than in the V-detour test. Those behavioral tests were recorded and analyzed using video-tracking software EthoVision® XT 11.5.

The Materials and Methods chapter is well designed. It includes the scheme of experimental design applied to the study, a table with doses of LPS and time of administration, six schemes and many photos of the experimental facility, and schematic representations of particular behavioral tests. Information and experimental diagrams are excellent sources of information about conducted research and tests. The photos show how much effort it took to conduct behavioral tests on sheep in the sheepfold. The chapter ends by describing blood sample collection to determine the cortisol, IL-6, and TNF-α levels performed for Experiment 3. Proper statistical tests were used to analyze data.

The Results chapter lists data in 43 figures and two tables. Results are divided into nine subchapters, and they demonstrated that behavioral tests used in experiments effectively showed subtle behavioral changes in lambs after LPS treatment in pregnant ewes.

After 14 pages of Discussion, the thesis is summarized with seven conclusions. LPS treatments in pregnant mothers induce MIA and affect offspring behavior. The developed animal model—sheep—can be used as a model of neurodevelopment disorders. Furthermore, video-tracking software can be successfully implemented in the study of sheep behavior, improving the reproducibility of the data obtained.

The Bibliography consists of 339 cited papers in English, with 34% of citations coming from the last 10 years.

As I mentioned at the beginning, I consider the PhD thesis topic very important because of the use of sheep as a research model. As a reviewer, I would like to address some questions/remarks to the Author:

- I am interested in the dose of LPS you used. A few years ago, in our study, I administered LPS in a dose of 400 ng/kg of body weight to ewes to create inflammation. You used a much higher dose of 1.2 µg/kg b.w. What was the reason for using such a high dose of LPS?
- Why did you collect blood and analyze some inflammatory factors only for Experiment 3?

- How you mated ewes with ram? Did you synchronize ewes before mating? Was it harem mating?
- Please explain what you mean by assignment to either experimental group was “pseudo-random”?

Conclusion

In summary, submitted for evaluation, PhD thesis, written by MS Wiesława Florek, contains many interesting results, which markedly increase our knowledge concerning sheep as a model of neurodevelopmental diseases and the use of the automatic video-tracking system for behavioral analyses.

Conducting the studies with so many sheep and with quite difficult behavioral tests required the Candidate to use a variety of experimental procedures and different research techniques and an enormous amount of work in the sheepfold. It should also be underlined that MS Florek has confirmed an extensive knowledge of the scientific literature on the research subject, reflected predominantly in a good review. I must highlight that all my remarks, presented during the thesis analysis, do not diminish the scientific significance of the work.

To conclude, MS Wiesława Florek's dissertation fulfills all the requirements for a PhD in zootechnics and aquaculture discipline. Therefore, I support applying to the Scientific Council of the Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences in Jastrzębiec for further processing of the PhD procedure.

I certify that the doctoral dissertation submitted for evaluation meets the requirements set out in the Act of March 14, 2003 on academic degrees and titles and on degrees and titles in the field of art (Journal of Laws of 2003, No. 65, item 595, as amended). I request the Scientific Council of the Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences in Jastrzębiec to admit Wiesława Florek, M.Sc., to further stages of the doctoral process.

Signed by /
Podpisano przez:

Dorota Anna
Zięba-Przybylska

Date / Data:
2024-11-03 14:22

Kraków, November 1, 2024

Dorota Zięba-Przybylska