

28-11-2023

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znak sprawy:

REVIEW OF DVM MARTYNA WOSZCZYŁO'S PhD DISSERTATION

BACKGROUND

DVM Martyna WOSZCZŁO graduated as vet in 2014 at the University of Environmental and Life Sciences, Wrocław Poland. Her professional experience includes serving as vet in general practice (General veterinary practice internship at Small Animal Veterinary Clinic – Dr Leonard Gugala), followed by a period working as a vet clinician in general practice (FYZJO WET – Dorota Strugala – Wrocław). DVM Martyna WOSZCZŁO appears to be strongly interested in the field of behaviour and behavioural medicine. During the years 2016-2018, she works as Animal Behaviour Training Specialist (Twoja Łapa Marzenna Woszczyło, Wrocław). From August 2017, up to now, she will be appointed as lecturer in three different institutions (PET IDEA Martyna Woszczyło Wrocław; Centre Of Applied Pet Ethology COAPE Polska Katowice; Polish Academy of Zoopsychology and Animal Therapy Cracow; Tischner European University Cracow) and as Specialist at Wrocław University of Environmental and Life Sciences Wrocław. In 2017, DVM Martyna WOSZCZŁO has followed a training period "Introduction to the physiology of chemical communication" at the IRSEA (France).

DVM Martyna WOSZCZŁO has published various papers in continuing education journals and presented continuous education lectures about behavioural medicine, in Poland.

DVM Martyna WOSZCZŁO has participated in different research programs:

National Science Centre (Poland) November 2021 –November 2024

"What Does Viral Infection Smell Like? Analysis of Volatile Organic Compounds and Their Detection Potential in Cell Line Models and Mouse Models."

Co-investigator in an NCN *Sonata* project no. 2020/39/D/NZ6/03185

National Science Centre (Poland) October 2017 – May 2023

Mechanisms of semiochemical communication in canines in the context of sexual behavior: the research with the use of the domestic dog (Canis familiaris) as a model species

Researcher in work conducted under grant no. UMO-2015/17/B/NZ8/02411

Dr Seidel Foundation March 2019 – June 2022

Dog smell preferences. Do dogs have their favorite scents?

Translational study in partnership with a commercial partner, financially supported by the development activity funds assigned to the Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Poland. The APC/BPC is co-financed by Wrocław University of Environmental and Life Sciences and by University of Life Sciences in Lublin.

Kokocińska A, Woszczyło M, Sampino S, Dziecioł M, Zybała M, Szczuka A, Korczyńska J, Rozempolska-Rucińska I. *Canine Smell Preferences-Do Dogs Have Their Favorite Scents?* *Animals* (Basel). 2022 Jun 8;12(12):1488. doi: 10.3390/ani12121488. PMID: 35739826; PMCID: PMC9219509.

Polish National Revenue Administration and University of Life Sciences in Wrocław March 2020 – April 2021

Disease Detection Dogs Project

The DDDProject aims to harness the unique olfactory capabilities of dogs to detect diseases in humans, including cancers, diabetes, and COVID-19. The project seeks to establish a specialized training methods for this purpose, allowing dogs to provide alerts for medical emergencies. In collaboration with The National Revenue Administration and the University of Life Sciences in Wrocław, a pilot study was conducted, training dogs to detect Sars-CoV-2. This training achieved an impressive 95% detection efficiency, emphasizing the potential for dogs in early disease detection efforts.

Project website: <https://dddproject.eu/en/>

DVM Martyna WOSZCZLO has also participated in different research projects with the team led by Prof Michał DZIECIOL, about the approach to dogs' sexual chemical communication. She has contributed to various papers published by this research group.

THE DISSERTATION

The dissertation presented by DVM Martyna WOSZCZLO title is "Research on selected aspects of semiochemical communication in dogs in the context of sexual behavior".

The dissertation begins with an introduction presenting the scientific context of the research. This introduction is divided into six different parts:

- Reproductive cycle of the female of domestic dog (*Canis familiaris*) in the context of intraindividual communication.
- Canine communication in context of chemical signalling.
- Canine olfactory system and its role in canine reproductive behavior.
- Can we teach dogs to detect pheromones?
- Pheromones.
- Canine sex pheromones

The introduction is followed by a paragraph defining the aim of the study. DVM Martyna WOSZCZLO will then introduce her personal work with a presentation of the 4 publications included in the dissertation. Each paper is summarized, and this presentation leads to preliminary conclusions.

The four papers are not presented in chronologic order, but according to their significance in the evolution of the research and its conclusions.

Paper 1 (pp 26-38): The role of urine in semiochemical communication between females and males of domestic dogs (*Canis familiaris*) during estrus. (*Animals* 2020, 10, 2112; doi: 10.3390/ani10112112)

Paper 2 (pp 57-71*): Urinary proteins of female domestic dogs (*Canis familiaris*) during ovarian cycle. (*Vet. Sci.* 2023, 10, 292. <https://doi.org/10.3390/vetsci10040292>)

Paper 3 (pp 39-56*): The influence of manual semen collection in male trained dogs (*Canis familiaris*), in the presence or absence of a female in estrus, on the concentrations of cortisol, oxytocin, prolactin and testosterone. (*PLOS ONE* <https://doi.org/10.1371/journal.pone.0278524> February 2, 2023)

Paper 4 (pp 72-83*): The case of atypical sexual attractiveness in a male domestic dog – A case study. (Animals 2021, 11, 3156. <https://doi.org/10.3390/ani11113156>)

*the pagination is wrong and does not follow the order of presentation of the papers 2 to 4. It would be good to edit it to make the reading easier.

Comments about the introduction: the introduction is well written and presented, and the list of references reflects the current knowledge in the field in an acceptable way. In the first paragraph, describing the reproductive cycle of the female dog, the author states that female dogs secrete attracting pheromones, during proestrus and estrus. This statement is not supported by any reference, despite its crucial role in the following part of the dissertation. This lack of citation must be edited.

In the paragraph related to pheromones, the wording is sometime missing precision, the author writes that dogs detect pheromones “through their sense of smell”. One may expect a more precise wording and especially a mention of the vomeronasal or accessory olfactory system, that is cited and described later.

The transition from a paragraph to another one is sometime difficult to understand. An example is related to the end of the paragraph about “Socialization”, that ends with the citation of Overall, 2013 and followed by “Sexual reproduction in dogs is believed...”. It is difficult to understand this transition. In this paragraph about sexual reproduction, the author mentions the word “semiochemical”, that is never defined anywhere else. It should be done for a better understanding of the introduction and following pages. On page 11, the first paragraph discusses the difference between pheromones and semiochemicals, but there is never any clear difference proposed by the author. Using the classification of semiochemicals by C. Kost in Encyclopedia of Ecology (2008), would help to clarify this part of the dissertation.

Page 11, in the paragraph about the canine olfactory system, some wording would deserve to be improved. The author writes that “Olfactory glands produce secretions to dissolve odorants and protect the neuroepithelium”. This is obviously the role of the OBPs and they do not “solubilize” but carry the odorants, that, in majority, are lipophilic compounds. To go through the olfactory mucus, those odorants need carriers, and this is the probable role of the OBPs. The word solubilize has another meaning in chemistry and should not be used in this context. Moreover, a short discussion about the lipophilic nature of most odorants would have been interesting to introduce the Paper 2.

Again in this paragraph, when DVM Martyna WOSZCZLO mentions that an olfactory neuron expresses a single kind of ORs, it would have been beneficial for the understanding of non-specialist, to illustrate the concept of chemotopy (B.A. Johnson, 2007).

Page 12, about the V2R, the author cites the absence of functional genes encoding for this type of receptors in dogs, and concludes (citing a controversial paper from McGlone, 2022) that it could be related to the regression of the VNO, as a consequence for domestication. It is regrettable that the author does not cite Shi (2007) or Dulac (different papers) and many others, who have published abundant literature about this question. To summarize, this type of receptors is absent in most mammals, with the exception of Monotremes and Rodents. There is no evidence that such receptors are present in wolves, the only way to support McGlone’s assertion. On the contrary, the presence of sensory cells all along the medial side of the VNO in dogs, as published by Salazar, is clearly supporting the contrary. According to the papers published by Dulac, the V2R receptors, that have a large

extramembranary domain of binding, are supposed to bind proteins. This work would be of high interest to discuss the results of Paper 2.

For page 12, when speaking about the "accessory olfactory system", I would recommend to use the concept of "vomeronasal system" proposed by Trottier.

On page 13, in the first paragraph, it would be better to avoid the wording "chemical particles" to describe the chemical signals that will be led to VNO cavity during tonguing.

The title of the next paragraph "Can we teach dogs to detect pheromones?", raises serious questions. As described in the literature cited by DVM Martyna WOSZCZLO, the axons of the sensory cells of the VNO, have no connection with the cortical areas (on the contrary to the olfactory system). It is very difficult to understand how the author could imagine training dogs to detect pheromones. According to Slotnick et al (2010) who described the role of the main olfactory system to activate the VNO system, this activation seems to be poorly controlled by any kind of cortical area.

On pages 14 (last paragraph "Pheromones") and the first part of page 15, one may regret, again, the absence of clear definitions for the different categories of semiochemicals (see my comment on previous page). The author also cites the concept of "interomone" proposed by McGlone but missing scientific background. It is the role of researchers to have a critical reading of literature.

In the paragraph about "canine sex pheromones", the author comments the real role of methylparaben as a putative sexual pheromone in dogs. It would be interesting to cite the massive use of this compound in chemical and especially cosmetic industry, as a preservative. This molecule, currently rejected by the authorities and consumers, for its hormonal effects in both humans and animals, may have been identified in female dogs' urine, consecutively to a contamination. This example of a false identification of pheromone compounds, illustrates the critical need of very precise and clean sampling to ensure the possibility to identify composition really produced by the animals.

Pages 16 and 17, it would be better to avoid imprecise wording like "thought to play" or "are believed".

Page 18: The author only works on urine, but there is no clear evidence that it is the only source of sexual pheromones. Other studies suggest that sebaceous secretions (notably controlled by prolactin and steroid hormones) play a role during sexual communication (McDonald et al., Social odors in mammals). One may suggest that looking only after urine, prevents the possible detection of active secretions.

Page 24, in the conclusions, DVM Martyna WOSZCZLO proposes 3 main results, between which "Proves the presence in the canine urine specific nonvolatile compounds (proteins), which detected by specific behavior ...". According to the results presented in Paper 2, it is difficult to support this assertion. The results published in this paper show that a range of proteins are observed in urine during estrus. This phenomenon is extremely interesting but the role of those proteins is not determined by the results of the study. The absence of functional genes encoding for V2Rs, in dogs as in most mammals, makes poorly probable that those proteins are the ligands activating the VNO of the males. On the contrary, their 3D structure, showing the presence of hydrophobic cavities, leads to the hypothesis of carriers and/or chaperones. It would be interesting to study the docking of some of the odorants identified by the author and her co-authors.

The following part of the dissertation is made of papers published in different peer-reviewed journals of scientific quality. Being published after peer-reviewing, those papers are proven to be of scientific quality. The statements published by the different co-authors of the papers, prove that DVM Martyna WOSZCZLO has played a major role in this research. This work includes the conception of the research, the management of the practical work to acquire the data, the analysis of the data, the review of the literature and the writing of the papers. In addition, all the research has been managed in cooperation with different other scientists, proving this way the capability of DVM Martyna WOSZCZLO to work inside teams. This is a determining capability for a scientist.

In conclusion, the manuscript of this dissertation describes highly valuable research in the field of chemical communication in dogs in the context of estrus. This manuscript needs some edition to reach the quality that such research report deserves. Anyway, this is an important work that will lead to a significant improvement in the understanding and management of canine reproduction and sexual behavior. The candidate, DVM Martyna WOSZCZLO, has presented a doctoral dissertation that reaches the appropriate scientific level. She has demonstrated her ability of independent scientific reasoning and thinking, as well as her ability to conduct research, but also to present it by mean of scientific publications in peer-reviewed journals.

To sum up, I state that this dissertation is a highly valuable amount of scientific results, that broaden our knowledge, and with outstanding results that will have very significant applications.

Therefore, I believe that the doctoral dissertation meets the conditions set out in art. 13 of the Act of March 14, 2003 on academic degrees and titles and on degrees and titles in the field of art and is the basis for conferring a doctoral degree.

Therefore, I submit the application to the Veterinary Discipline Council of the Wroclaw University of Environmental and Life Sciences to accept a job and admit a veterinarian Martyna Woszczylo to further stages of the doctoral procedure.

A handwritten signature in black ink, appearing to read 'Patrick Pageat', with a stylized flourish at the end.

Professor Patrick PAGEAT

DVM, MSc, PhD, Dipl. ECAWBM, Hon. Dipl CLECVe