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Annex No. 11 to the MU Directive on Habilitation Procedures and Professor Appointment Procedures

HABILITATION THESIS REVIEWER'S REPORT

Masaryk University	
Faculty	Faculty of Science
Procedure field	Animal Physiology
Applicant	MVDr. Martin Anger,CSc.
Applicant's home unit, institution	Masaryk University, Faculty of Medicine
Habilitation thesis	Control of chromosome segregation in mammalian female meiosis
Reviewer	doc. Alexander W. Bruce, Ph.D.
Reviewer's home unit, institution	University of South Bohemia in Ceske Budejovice, Faculty of Science.

The focus of Martin Anger's research career to date has been committed to furthering our understanding of the collective processes that regulate the precise segregation of replicated chromosomes into resulting daughter at cell division. Specifically, although not exclusively restricted to, how these processes can result in the reductive divisions of meiosis I of the female gamete, resulting in a fertilisable egg cable of sustaining subsequent embryonic development. Accordingly, the work contained within his submitted habilitation thesis summarises his research in the period following the award of his Ph.D. in 2001, up to that conducted in his own independent laboratory in the present day.

During these 18 years, Martin has successfully completed two post-doctoral positions, receiving excellent training in the laboratories of world renown and recognised leaders in the field (namely, Prof. Richard Schultz at the University of Pennsylvania in the USA, for four years; and Professor Kim Nasymth, mainly at the University of Oxford, for three years), before making the transition to independent research group leader at the Czech Academy of Sciences' Institute of Animal Physiology and Genetics in 2008. He later moved to join CEITEC in 2011, becoming director of the CEITEC Organisation Unit at the Veterinary Research Institute in Brno in 2015 and head of the Core Facility for Cellular Imaging from 2016. He has been affiliated with the Department of Histology and Embryology in the Faculty of Medicine at Masaryk University since 2013.

A search of the PubMed database reveals Martin has been an author to 28 articles (including one review article) since 2001, although a small number of articles appear to stem from his Ph.D. research. However, from this total of 28 articles it is noteworthy that only 12 are directly referenced in this habilitation thesis. This in my opinion serves to highlight the strong nature of Martin's candidature, given he is able to be in a position to be selective about which studies he has chosen to put forward for consideration; i.e. adopting a quality versus quantity-based approach, which does him great credit. From the submitted 12 publications, one derives from the laboratory of Prof. Schultz, four from Prof. Nasymth and the remaining seven from his own independent research team, amassing 440 non-self-citations to date. I as opponent particularly respect the emphasis that Martin has placed on prioritising his own independent and self-corresponded research programme and take it as further evidence of his successful transition from accomplished post-doctoral researcher to proficient group leader; an obvious skill and characteristic for any prospective habilitation candidate. This latter point

is further strengthened by the fact Martin has successfully competed for a number of prestigious grants that have facilitated this effective transition and establishment of his research group; including the prestigious J. E. Purkyne fellowship, EMBO installation and Marie Curie reintegration grants.

The structure of the presented thesis follows a conventional format with a short introduction of the life cycle of the mammalian oocyte, passing into four structured sections detailing specific aspects of the meiotic maturation of oocytes once they are periodically recruited back into the reproductive cycle after prolonged arrested development. The thesis is then concluded with a section highlighting future perspectives and pertinent research questions still to be resolved. The four structured sections specifically relate to; **i.** the inherent nature of mammalian oocytes to exhibit enhanced rate of aneuploidy (compared with other cell types), **ii.** how the segregation of chromosomes is regulated at the molecular level during meiosis, **iii.** how the meiotic spindle assembly is regulated, and **iv.** the meiotic regulation of the Anaphase Promoting Complex/ Cyclosome (APC/C). Each section provides a concise and digestible summary of the current state of the appropriate knowledge and elegantly integrates the contribution made by the studies of which Martin was an author. Additionally, I positively acknowledge the fact that Martin is able to address the subject of mammalian oocyte maturation in a holistic manner and has not allowed himself to be compartmentalised to only studying a single aspect or molecular process. This is exemplified by the fact he has made significant contributions in each of the sub-sections I have listed and that in his independent research, he has also branched out to investigate how the meiotic mechanisms he has helped to elucidate may also contribute to the cleavage divisions of preimplantation mammalian embryos.

In relation to the described contributions Martin has made in this thesis, I have a series of questions, that are articulated below. I would however first like to highlight that in my opinion each of the contributing articles have been extremely well conceived and executed; they are all very high-quality studies. Whilst, it could be argued that it is difficult to assess Martin's direct contribution to those studies conducted in the laboratories of his eminent post-doctoral supervisors (representing the minority of this thesis), the fact the same hallmarks of careful, deliberate and hypothesis driven, and not over-interpreted, research are also present in the work emanating from his own group, serves to illustrate his commitment to rigorous scientific method. Moreover, the fact that he combines his own research programme with running the Core Facility for Cellular Imaging at CIEITEC/MU, assisting his colleagues with their own research according to this appropriate method is also highly noteworthy. Indeed, in my opinion there are very few other labs in the world that are able to surpass the quality and usefulness of the time-lapse imaging of early mammalian developmental events (not just restricted to oocyte maturation) achievable in Martin's group.

Questions to be asked at the juncture.

In summary, it is of my firm and convicted professional opinion that Martin Anger amply fulfils the requirements for habilitation at Masaryk University and I am pleased to be able to make that recommendation to the relevant committee of the university.

Reviewer's questions for the habilitation thesis defence (number of questions up to the reviewer)

1. Are there any overarching theories why the rate of aneuploidy is so comparatively high within the mammalian female germ line, even in young individuals, compared to other somatic cell types? What could be the evolutionary pressures at work, particularly within primate species (such as ourselves) that invest relatively large amounts of reproductive capital in rearing small numbers of offspring? On a related note, are there any indicators that may functionally explain why oocytes

from young pigs exhibit relatively high rates of aneuploidy that do not significantly increase with maternal age but humans and mice start from a lower base that does increase with maternal age?

2. As the formation of meiotic spindles in mammalian oocytes occurs in the absence of centrosomes (relying on chromosome proximal MTOC activity) and early cleavage stage embryonic mitotic spindle assembly is governed by paternally inherited centrosomes, is it known if the pre-blastocyst stage mitotic divisions of mouse preimplantation embryos (that lack paternally inherited centrosomes) invoke spindle assembly mechanisms directly analogous to oocyte specific meiotic spindle assembly? Incidentally, are there any other known examples of early mammalian embryo mitoses, other than in mice, that are governed by acentrosomal spindle assembly and is it known how centrosomes are derived *de novo* in the cells of the mouse blastocyst?
3. In the discussion of your studies investigating the somewhat weak strength of the meiosis I spindle assembly check point (SAC) in mouse oocytes (often permitting segregation of non-congressed chromosomes), you provide a rational argument that this may be related to the atypically large volume of the oocytes effectively diluting the concentration of SAC components that regulate a fixed number of kinetochores (compared to other smaller somatic cell types). Is there any direct evidence that SAC components are less concentrated in cytoplasm of oocytes compared to somatic cells? Additionally, is there any evidence for increased compensatory expression of SAC components in oocytes versus somatic cells? You mentioned attempts to investigate the relationship between SAC activity and oocyte volume have been inconclusive, are you able to expand on this point and explain how you might design an experiment to help resolve the ambiguity?
4. As a follow up question, given there is an extraordinary high rate of aneuploidy observed in the oocytes of sterile *M. musculus* and *M. spretus* hybrid, but a surprisingly normal level of SAC activity, can you speculate what might be the underlying mechanism(s) responsible for the misaligned and chromosomal congressional defects and non-disjunctions you observe? Is there any evidence for differential expression of key genes identified in *M. musculus*, that could be different *M. spretus?*

Conclusion

The habilitation thesis entitled "Control of chromosome segregation in mammalian female meiosis" by MVDr. Martin Anger, CSc. **fulfils** requirements expected of a habilitation thesis in the field of Animal Physiology.

Date: 29th October 2019 Signature: doc. Alexander W. Bruce, Ph.D.