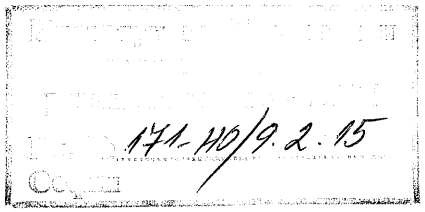

To the Scientific Jury of IBIR-BAS

(Reg. No. 18/14.01.2015)

Expert Statement concerning PhD candidate Desislava Vasileva Abadzhieva and her PhD thesis with title: *"Estimation of the Spirulina platensis and Vemoherb-T supplementation effect on the reproductive parameters of female rabbit"*

I have come to know the candidate Desislava Vasileva Abadzhieva through the ReProForce FP7 project. She has visited Wageningen University twice, at the beginning of her PhD project in 2010 and last year, to perform some immunohistochemical experiments for her thesis. Between het first and second visit to our lab the candidate has changed from a relatively insecure young scientist into a dedicated scientist with a clear focus on what she intends to do. She has developed into an independent hard working investigator, who very well knows to find her way in a strange lab, eager to acquire as much knowledge as possible in a relatively short period of time. This made her second visit to Wageningen very successful, as the immunohistochemical data of her thesis show.

The subject of the thesis of the candidate is novel and intriguing. Scientists become more and more aware of the possible positive effects of food additives in prevention of disorders related to for instance overweight/obesity, cardiovascular disease and cancer. Much less attention is paid to the possible effects of these additives to male and female reproduction. A quick screen through PubMed shows that only a limited number of studies have investigated the consequences of *Spirulina platensis* and *Tribulus terrestris* supplementation on female reproduction. The focus in these studies is on the effects of reproduction in general and pregnancy outcome, ignoring direct effects of these compounds on what happens before an oocyte is fertilized, namely follicular development. The study described in this thesis is as far as I know, the first study that analysis possible effects of *S. platensis* and *T. terrestris* on follicular development and possible effects of (pre)pubertal treatment on follicular development in ovaries of the female offspring of the treated mothers. The selection of the rabbit as model animal is interesting, as not much research has been performed on rabbit reproduction.

In the study described in the thesis of Desislava Vasileva Abadzhieva different methods are used to analyze the effects of *S. platensis* and *T. terrestris* on ovarian follicular development. First of all, body weights and ovarian weights are analyzed. Secondly, the ovaries of mother does and their female offspring are investigated in order to determine whether the treatments affect follicular development. This analysis shows that indeed *S. platensis* and *T. terrestris* have a positive effect on the follicular composition in the ovary of female rabbits. The effects of S. platensis being somewhat more pronounced, especially in the female offspring than *T. terrestris*. The fact that *S. platensis* has such a significant effect on follicle numbers in the female offspring that have not been exposed to the algae themselves is very interesting.
Next the candidate investigates whether there are differences in the expression of two genes that have been implicated to play an important role in recruitment of primordial follicles in the growing pool and further follicular growth, using RT-qPCR, an up to date technique to acquire this type of

information. By isolating oocytes and cumulus granulosa cells separately, the candidate is able to investigate gene expression at a cellular level. The candidate is the first to show that *S. platensis* treatment leads to increased expression of Gdf9 in oocytes of mother does. This change in Gdf9 expression is not transmitted to the offspring. In contrast, Bmp15 expression was significantly increased in both oocytes and cumulus granulosa cells of mother does, and this change in Bmp15 mRNA expression in oocytes was transmitted to their female offspring, implicating the involvement of a possible epigenetic mechanism.
T. terrestris treatment also significantly affects Gdf9 and Bmp15 expression in oocytes and cumulus granulosa cells in mother does, while a trend is observed in the female offspring. As changes in gene expression not always result in changes in protein levels, the candidate further investigated the presence of Gdf9 and Bmp15 at the protein level using immunohistochemistry. This technique has the advantage that proteins can be identified at the cellular level. By applying an up to date image analysis technique the candidate shows for instance that indeed in the *S. platensis* fed mother does the increase in Gdf9 mRNA levels in oocytes results in an increase in Gdf9 protein in oocytes of developing follicles, while like at the mRNA levels, no effect is observed in the female offspring. The results of these two analyses are however not always as straight forward. In some cases changes in mRNA and protein levels of Gdf9 and Bmp15 do not follow the same pattern, suggestive of a possible effect of *S. platensis* and *T. terrestris* at the posttranscriptional and posttranslational level.

As mentioned before, this investigation is the first that studies the effects of the feed additives *S. platensis* and *T. terrestris* at the ovarian levels. The data presented in this thesis show that follicular development and oocyte quality in relation to treatment with feed additives like *S. platensis* and *T. terrerestris* are very important parameters that should be included in the analysis of possible effects on reproductive health. The thesis shows some interesting data and forms a sound basis for further research, as there is still a lot of work to be done, such as for instance the investigation of effects on follicular degeneration. Can these feed additives prevent follicles from undergoing atresia and in this way prolong reproductive lifespan? This knowledge is very important for a practical application of these feed additives in animal breeding.

My final decision is positive / negative to reward the PhD candidate with the PhD degree.

Date:
February 9, 2015

Signature

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