

**Report of the Short term placement visit of Joanna Wyszynska-Koko from Institute of Genetics and Animal Breeding, Polish Academy of Science in Jastrzēbiec, Poland, to Marinus te Pas, Animal Sciences Group of Wageningen University and Research Centre, Lelystad, The Netherlands.**

May 14, 2007 – July 6, 2007.

**Joanna Wyszynska-Koko**

**Aim:** to learn microarray hybridisation and analysis. During the visit the aim was realized by working on the SABRE WP6 project. Some aspects of the project and the work are described below.

**Conclusion:** The aim of the visit has been realized.

**Some information about the work realized during the visit:**

*Introduction*

Poor fertility results in high replacement rates and, consequently, has major implications for animal welfare and sustainability of dairy industry. The SABRE WP6 project investigates expression profiles (i.e. microarray analysis) of diverse brain areas of cattle in relation to oestrus and fertility.

The expression of genes in Ventral Tegmental Area (VTA) of cows was analyzed. VTA is the group of neurons in a midbrain, being considered as a pleasure system and reward circuit. As such, VTA is important in a behavioural motivation aiming at actions needed for vital functions as eating and reproducing. In this way VTA is involved in total neurophysiologic and behavioural background of reproduction and can be responsible for differences in reproduction efficiency, as the problems with fertility concerns also weakly shown sexual behaviour during oestrus.

*Materials and Methods*

**Animals:** Cows showing high and low oestrus behaviour were euthanized and the various parts of brain were sampled in time of one hour after the death of animal and stored in liquid nitrogen. Basing on behavioural data, 30 cows of highly and lowly expressed oestrus behaviour were chosen. RNA was isolated from the VTA samples, according to the method used for microarray analysis. For further analysis, the samples were also grouped according to the day of oestrus cycle, what generated four groups of samples: 1) cows of highly expressed fertility traits, in day 0 of oestrus cycle, 2) cows of highly expressed fertility traits, in day 12 of oestrus cycle, 3) cows of weakly expressed fertility traits, in day 0 of oestrus cycle, and 4) cows of weakly expressed fertility traits, in day 12 of oestrus cycle. The RNA samples were pooled according to the groups and used for microarray analysis. The experiment was set to compare total expression of genes in VTA between all four groups, according following comparisons: 1) high fertility, day 0 vs. high fertility, day 12; 2) low fertility, day 0 vs. low fertility day 12; 3) high fertility, day 0 vs. low fertility day 0; and 4) high fertility, day 12 vs. low fertility, day 12.

**Microarrays:** Bovine whole genome oligo microarrays were used. On each slide 20 000 70-mers corresponding to bovine genes were printed. Because there were not any repetitions of genes on the slides, every particular hybridisation were repeated twice.

*Results*

During doing the hybridisations, some methodological problems were solved so the signal of spots after changing the procedure was more intense, with proper background level. After doing the hybridisations, the slides were scanned. The images of microarray slides were elaborated using the GenePix software. The software analysis resulted with a list of genes with raw intensity data, which were used for normalisation analysis (normalisation individual slides, the software available on Bioinformatics website of ASG, Lelystad). After normalisation step, the data of particular comparisons were used for further statistical analysis, combining microarrays data (CombNormTxt), and the third step, significant analyses (SAMuG). Because the statistical analysis failed due to hardware problems of the bioinformatics, the analysis was repeated with Excel software. In this analysis the spots are considered regarding to their M, A and P-values. M value indicates the difference between the level of Cy5 and Cy3 labelled RNAs. The spots with  $-1.58 < M < 1.58$  (log<sub>2</sub> scale) were taken into consideration. They were also analyzed regarding significance of the M-value, which had to be  $P < 0.05$ . These analyses allowed to create a list of genes up and down regulated, comparing the mentioned groups.

Finally, the results were prepared for biological interpretation. The main step of it is a pathway analysis. Because in the time of placement the hardware problems in Statistics Department did not enable to do this analysis, it has been moved to the time of further cooperation, which was set up by this successful placement. Joint publications are in preparation.