Both clinical and subclinical mastitis causes big economic losses in the dairy farming and industry. However, the disease background, associated with inflammatory processes in healthy and infected quarters of udders are still not fully known. Although, there are several studies on the processes occurring in the healthy quarters adjacent to infected ones, however, researchers mostly used milk and/or milk somatic cells as a material for the analyses. Only a few analyses are performed on a secretory tissue. Additionally usually these are samples from artificially infected quarters of udders in order to obtain healthy quarter as a control to the infected ones as the material for the experiment. **The study aims to** determine the phenomena taking place in the dairy cows' mammary gland parenchyma with the prevalence of secretory tissue (ST) derived from quarter adjacent to infected with coagulase-positive (CoPS) or -negative (CoNS) staphylococci causing subclinical chronic infection (AHCoPS – quarter adjacent to CoPS, AHCoNS – quarter infected to CoNS) compared with quarters derived from the whole healthy udders (H – control group consisting of healthy quarters). This study will be a continuation of a running project.

Planned analyses: 1. Analysis of mRNA level and lncRNA (long non-coding RNA) profile in AHCoPS and AHCoNS vs. CoPS, CoNS and H (NGS), 2. Quality and quantity protein analyses in the same samples (MALDI-TOF), 3. Bioinformatic analyses of transcriptomic, proteomic and epigenomic results and lncRNA profile, 4. Statistical analysis to evaluate the relationships between mRNA, proteins, and lncRNA profiles. The results of the miRNA profile obtained during previous projects will be also analysed together, 5. Functional analyses of differentially expressed genes.

We expected a higher expression of cytokine genes in quarters adjacent to staphylococci infected than in H. However, it turned out that the expression of cytokine genes at the mRNA and protein levels in AHCoPS were similar as in H and lower than in CoPS, while in the opposite results were obtained for AHCoNS (expression was higher than in H). It was very surprising, especially that CoNS infections are usually classified as non-pathogenic. It is also suggested that cows with CoNS infection are less susceptible to other infections and also that CoNS infection does not impair milk yield and its component. Therefore it is very interesting to get knowledge on the exact processes occurring in AHCoPS and AHCoNS quarters as it is a routine that the milk from them is used for consumption if the cow is not treated with antibiotics (especially with subclinical CoPS or CoNS udder infections that are very often not diagnosed).

Hypotheses: 1. The processes occurring in the staphylococci infected quarters affect the physiology processes of healthy quarters, adjacent to the infected ones, influencing transcription or translation processes - the inflammation occurring in at least one infected quarter disturbs homeostasis in adjacent quarter, inducing the production of pro-inflammatory agents that damage the healthy tissue despite the lack of pathogen and lower their productivity; 2. Substances stimulating immunity may also penetrate the barrier between the quarters and induce the immune response in the uninfected quarter. It would mean that the experiments, which consider the uninfected quarter as a control for the neighboured infected quarter, are loaded with a significant error. Bovine udder quarters are still considered four separate glands.

All secretory tissue samples from each quarter of 50 Polish Holstein-Friesian dairy cow will be collected post-mortem and stored in -80°C. The samples are divided into five groups: (1) CoPS (N=10), (2) AHCoPS (N=10), (3) CoNS (N=10), (4) AHCoNS (N=10) and (5) H (N=10). The same samples will serve for total RNA and protein isolation for transcriptomic and lncRNA profile and proteomic analyses, respectively. Analyses planned in the project will be performed for at least 7, the best samples from each group selected based on the mRNA quality - with the lowest level of degradation and best RINs (RNA Integrity Number). The results from the currently run project (PRELUDIUM BIS 2) about miRNA profile and the results of the presented study regarding protein, mRNA levels and lncRNA profiles will be used for statistical and functional analyses to get very comprehensive results. It will allow for a broad view on the processes occurring in the bovine udders with at least one quarter infected with staphylococci. Thus, the full analyses of the phenomena occurring in the infected and quarters adjacent to them vs. the H will allow for obtaining a complete information on the changes occurring during inflammation both in infected and healthy quarters. Moreover, the results should clearly explain, if using uninfected quarters adjacent to infected ones as a control group for the infected is a proper method or is burdened with high bias. Next, it will be studied, if milk from the quarter adjacent to infected intended on the market is safe for consumers. The planned analyses would expand our knowledge and help us to understand the complex mechanisms of the disease and allow to draw correct final conclusions about the processes occurring in the whole mammary gland when at least one quarter is infected. Innovation of the project is related to the examination of the effects of bacterial infection in one quarter on the transcriptomic, proteomic, and epigenomic phenomena in quarter adjacent to infected.