Introduction/Background MicroRNAs (miRNAs) are small non-coding RNA molecules regulating gene expression that may have diagnostic potential by being associated with different diseases, including epithelial ovarian carcinomas (EOC). However, there is a lack of consensus how to accurately quantify miRNA levels, which hinders their implementation in diagnostics. Real-time qRT-PCR is often considered as the golden method; however, the results might be biased by various handling of missing data and normalization approaches. Only a few studies have been published to date on the identification of endogenous miRNA controls in EOC. Therefore, our aim was to identify stable candidates based on previously published- and three public miRNA-microarray datasets and verify their stability in a new cohort of EOC patients. Moreover, our goal was to compare different missing data and normalization approaches to investigate their impact on the results.

Methodology Following RNA extraction from formalin-fixed paraffin embedded tissues from 80 high-grade EOC patients, a custom designed panel of 48 miRNAs was investigated by RT-qPCR and analyzed by applying various strategies regarding missing data (a listwise/pairwise deletion, mean substitution, replacing non-detects with a Cp value of 40, multiple imputation), choosing stable endogenous controls (GeNorm, BestKeeper, NormFinder, the comparative ΔCt method and ReFinder) and normalization based on endogenous controls, spike-ins or global mean.

Results We identified 20 endogenous control candidates by combining miRNA microarray data analyses of four datasets and literature screening. Among these candidates, hsa-miR-101-3p, hsa-miR-191-5p, and hsa-miR-193a-5p were subsequently validated as most stable in 80 EOC patients. Moreover, we present how different approaches of data handling affect results, e.g. common practice of setting missing Cp values to 40 might lead to large (likely false) differences in miRNA expression between patients.

Conclusion Our data demonstrated the challenge of miRNA qRT-PCR data analysis and the need for standardization if comparison/conclusions across datasets are performed.