Evaluation of allograft quality before engraftment is becoming an important task in the realm of organ transplantation. Prediction of renal allograft outcome for making the final decision to do the transplant or not, is not a well-addressed issue (1). This necessity becomes more obvious as the shortage of organs has increased the acceptance rate of risky organs retrieved from ‘extended criteria’ donors (ECD) and donation after circulatory death (DCD) (2,3). These kidney allografts harbor more susceptibility to delayed graft function (DGF) and require more elaborate tools to preserve and predict the graft viability (2,3). Hypothermic machine perfusion (HMP) is not only a reliable preservation tool but also a novel method to investigate the allograft status (2,4). A key question is which parameters can be applied during HMP to predict the allograft quality. Currently, the accepted signs of kidney allograft viability are renal flow rate and vascular resistance measurement during HMP (5,6). Elevated perfusion resistance during HMP has been shown to be a risk factor for future graft failure. Additional parameter includes ex vivo assessment of oxygen consumption during HMP; higher oxygen consumption is positively correlated with better allograft function (2). Measurement of metabolomics activity in HMP perfusate using NMR (nuclear magnetic resonance spectroscopy) is another proposed method (7,8). Although some biochemical and ischemic injury markers have been specified in the renal allograft effluent, they are not sufficiently established since their role in allograft function prediction in vivo is yet disputable (5,6).

microRNAs (miRNAs) are small noncoding RNAs that regulate gene expression and control physiological and pathological processes (9). miRNAs are considered to be pertinent to kidney injury and promising novel biomarkers due to their stability in body fluids and their correlations with pathological processes. Cell-free or circulating miRNAs in perfused solution during HMP can also offer a valuable pre-transplantation biomarker for evaluation of organ viability (3). Gómez-Dos-Santos et al., identified a panel of circulating microRNAs (miR-144-5p, miR-486-5p, miR-142-5p, miR-144-3p) in perfusion fluid during HMP that was correlated with future DGF development (3).

Quantitative PCR (qPCR) is an accurate and sensitive method for quantifying circulating miRNAs, but harbors great limitation that originates from its normalization methodology considering the inter-individual variability (10). Cell-free miRNAs are subjected to a set of pre-analytical factors including the amount of the sample, the collection and storage conditions, and the efficacy of miRNA isolation and reverse-transcription (RT) that all can alter the total miRNA levels (10,11). The normalization of RT-qPCR data is essential to diminish these technical bias presented during the entire experimental process. Several methods have been developed and used to normalize circulating miRNAs that hinder the possibility of comparing results from different studies. In order to normalize RT-qPCR data, endogenous miRNAs (housekeeping genes) (12), exogenous synthetic oligonucleotides (spiked-in method) (13), and the geometrical mean of all the expressed miRNAs (3,13) are used, each having their own pros and cons.

microRNAs along with small nuclear and nucleolar
RNAs have been reported to be stable cellular housekeeping genes. Since these controls are affected by similar sources of variability during the experimental processes as other target genes, they remain reliable normalization genes. However, evidence indicates that these RNAs are highly variable and are not stably detectable in body fluids (14).

In order to overcome this issue, synthetic oligonucleotides have been used. Since synthetic oligonucleotides are added prior to miRNA isolation, they follow the same processes and experimental biases as endogenous miRNAs do. This normalization method eliminates the variability in RNA extraction and RT reaction and it is more accurate; however, it entails some limitations. Some technical variables and errors that are existed before these processes and even differ in several types of body fluids and diseases, cannot be normalized by this method. To solve this issue, a combination of endogenous and synthetic oligonucleotides is used (11). Gómez-Dos-Santos and coworkers concluded that the mean expression value presents a better normalization strategy for the evaluation of miRNAs in perfusion fluid during HMP (3).

Because of the present heterogeneity in normalization strategies, it is impossible to define universally accepted reference genes for circulating miRNAs quantification. Therefore, it is required to focus on standardizing methods to select case-specific reference genes. Identifying and validating the most stable miRNAs for each experimental setting and selecting them as reference genes seem to be the best normalization strategy so far. In situations where the data is larger, besides selecting the most stable genes, global mean value can be advantageously utilized since all the miRNAs used as reference genes will be considered as target genes.

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Footnote

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