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In silico reference genes evaluation for real-time qPCR in oocytes and embryos of buffalos

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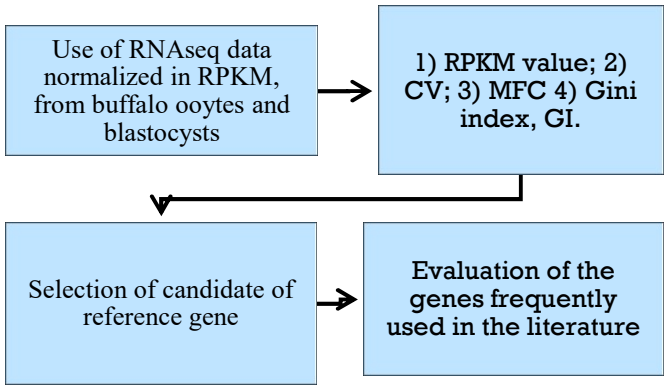
Introduction

- The use of reference genes is a simple and popular method to normalize data in Real-Time Quantitative Polymerase Chain Reaction (qPCR), so the selection of reliable reference genes is essential to correctly interpretation of qPCR results.
- Currently, in silico methodologies have been developed to identify new reference gene candidates, that are based on RNA-seq analysis, and performed to select genes which presents low variability in expression across tissues and experimental conditions and presents essential cellular functions.

Main Goal

- This study aims to identify new reference genes candidates in oocytes and in vitro blastocysts from buffalo using a combination of in silico methodologies.

Methods



- Reads Per Kilobase Million (RPKM). Then, using Excel and R studio software, genes were classified as stable expression among the samples considering the following criteria: 1) RPKM value; 2) coefficient of variation values, CV; 3) Maximo fold change, MFC (J et al. 2007. Plos One, 2:1-5); 4) Gini index, GI, which measures inequality in the expression levels of each gene between samples (Oet al. 2018. CellSystems, 6:230-240).

Results and Discussion

- As a result, of the total of 8,649 genes expressed in the RNA-seq of buffalo oocytes and embryos, 18 genes (NACC2, ZNF106, DDX28, MED4,THUMPD1, IRF2BP2, ELK1, QKl, EFTUD2, RAPGEF1, RAPGEF1, CCNYL1, PJA2, NUP58 , GOLT1B, TTC4, GNL3 and PRR14L) showed stable expression values and are considered candidates for reference genes.
- The 18 candidate genes were selected in all 4 methodologies used, as they presented: RPKM values >40 (moderate/high expression), CV <3%, MFC <2 and GI ranging from 0 to 1.
- Finally, the biological functions of genes were researched on the Genecards platform (https://www.genecards.org/) being related to RNA transcription and processing, protein regulation, protein folding and stem cell proliferative capacity, that is, they exert essential cellular functions
- In the second step, standard reference genes such as GAPDH, YWHAZ, SDHA, G6PD, B2M and A2M that presented CV >100%, MFC >2 and IG >4 were evaluated, thus it was considered that they did not exhibit stable expression between the samples analyzed
- The in silico methodologies listed here have already been used in the literature to select stable genes in humans, bacteria and fish and, in the present study, they were used in a combined way to increase the selection rigor.
- The reference gene selection is usually neglected in many studies that use a limited variety of genes considered standard, and, it is well know that these genes can show variations in expression level when compared between different situations and/or tissues.
- So the combination of in silico methodologies can be a useful selection tool as long as RNA-seq data are available and further validation by qPCR is performed, in order to confirm the gene expression values.

Conclusions

- In conclusion the 18 genes selected in this study are promising new gene reference candidates to be used in real-time qPCR assays, so this way to quantify gene expression in oocytes and buffalo embryos, but prior validation by qPCR is required.