Validation of 26a and let7 microRNA as early pregnancy markers in single cow blood samples, at day 28 after embryo transfer.

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Abstract

In this work we validated the previous scientific reports done in pooled samples by two different groups, over the miRNA expression in single blood from pregnant cows. Particularly we indagate the expression at day 21 after embryo transfer of let 7a, and 26a as well as 16 microRNAs (miRNA) family. By qPCR technique we demonstrated that let 7a, and 26a increase maximum 2.1 and 2.6 fold change respectively in pregnant vs non pregnant nulliparous heifers Fig1. We hypothesized that 26a and let7a miRNA are suitable for early embryo transfer viability at day 28 in single blood samples from nulliparous heifers. Moreover, this differential miRNA expression can be applied in the field as early blood pregnancy markers. We aimed to determine the differential expression of 5 microRNA let-7a-5p, 16-5p, 16-3p16-3p, 26a, and let-7a miRNA at day 28 day after embryo transplantation in single blood samples.

Materials and Methods Samples

Heifers were synchronized with the use of progesterone devices (CIDR) for five days. After 3 days when CIDR was removed, it was given an injection of Gonadorelin (GnRH).

Seven days after GnRH injection one IVF embryo was transferred to the uterine horn ipsilateral to the corpus luteum (CL).

Pregnancy diagnosis was performed 28 days after GnRH injection via conventional rectal sonography.

10 mL of blood was collected in K2 EDTA Vacutainer tubes (Becton Dickinson, USA) by tail venipuncture, using 18G needles (Becton Dickinson).

Ten pregnant and 10 non-pregnant heifers on day 21 after GnRH injection were used in miRNA analysis. In order to avoid blood hemolysis the serum was separated by centrifugation at 1600g at 4 °C for 10 minutes and then stored at -80 C the same day of the blood sample was taken.

Results

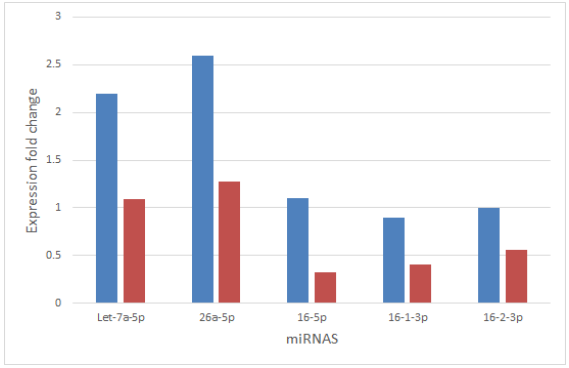

Figure 1. Relative fold expression change for miRNA normalized with RNU6 miRNA between pregnancy and failed embryo transfer pregnancy cows. Expression fold change was calculated with delta delta Ct methodology by comparing pregnant vs nonpregnant data at day 28. RNU6 is a small nuclear ribosomal for normalization of miRNA with relative quantification. In blue, experiment 1 N=10. In red, experiment 2 N=10.

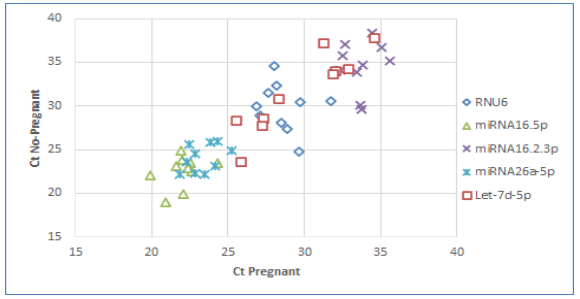

Figure 2. Threshold values for 10 pregnancy vs 10 no-pregnant cow blood samples cow. The detection of the miRNAs was performed by qPCR reactions with specific miRNA primer.

- Data support that UNR6 expression is an excellent gene to normalize the fold in miRNA expression. This is in accordance with previous forensic analysis studies (Sauer et al. 2014) and quantification of miRNA for prostate cancer diagnosis (Schaefer et al. 2010).
- In this work we validated that let-7a and 26a miRNA expression increase 2.2 and 2.6-fold respectively between pregnant and failed embryo transfer (open) samples at day 21 when the pregnancy status was checked by sonography.

References

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Discussion

In humans during tuberculosis infections **miR-26a** and this is a negative regulator of transcriptional coactivator p300, a component of the IFN-γ signaling cascade (Ni et al. 2014). In addition, under virus infection miR-26a has been reported to increase the phosphorylation of STAT1 and promotes type I IFN signaling that inhibits viral replication (Zhang et al. 2019).

Clearly at an early stage of pregnancy as well as infections respond miR-26 rapid increase in blood and tiger immunological response. The correlation of pregnancy and immunological modulatory effect under miRNA modulation demand attention and further investigation.

In vitro data shows that **Let 7 a/b** family expression tiger immunological response. Blocking CTLA-4 expression, a key protein receptor that downregulates immune responses (Yu et al. 2019). And, plays an important role in cell signaling and trophoblast adhesion (He et al. 2019). In addition, let-7 miRNAs family inhibit the activation of NF-κB signaling and expression of proinflammatory cytokines *in vitro*, (Zhao et al. 2018).

The data shows a variation between experiments, see figure 1 and 2. The variation of the data must be explored with a large number of samples and must be evaluated by the biological rationale of this fold change miRNAs specimens.

Taking all this data into consideration miRNA 26a and let 7a can be used as early pregnancy markers in cattle.

More work should be done in order to elucidate the relationship between these upregulated miRNAs and the possible immunological response in cattle. Better understanding of these pathways in early pregnancy in cattle could allow better control of the reproduction and management of cattle.