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Original Article

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Abstract

MicroRNAs (miRNAs) can post-transcriptionally regulate gene expression and play an important role in the development and function of organs and cells in cattle. This study identified novel and differentially expressed miRNAs in the maternal placenta of Holstein cattle with retained fetal membranes (6 cows for each group). The study found 4,596,223 reads that matched the bovine genome in the normal maternal placenta library (5,930,853 reads in total), and found that a total of 4,270,190 reads were present in the retained fetal membrane maternal placenta library (5,367,946 reads in total). In the present study, 36 novel bovine miRNAs were identified in the normal and retained fetal membrane library and 33 novel miRNAs in the maternal placenta libraries. It was discovered that 69 known miRNAs were significantly differentially expressed between the two samples (P<0.05), with 33 up-regulated retained fetal membranes and 36 down-regulated maternal placenta miRNAs. Real-time quantitative PCR analysis was used to verify known miRNAs. Notably, six miRNAs (down-regulated: bta-miR-423-5p, bta-miR-181a, bta-miR-185; up-regulated: bta-miR-411a, bta-miR-31, bta-miR-424-5p) were significantly highly expressed and exceeded 1.2-fold change. These known miRNAs may be important for retained fetal membranes in cattle. Possible causes of the fetal membrane retention were discussed with a bioinformatic analysis of the differentially expressed miRNAs.

Keywords: maternal placenta, miRNA, retained fetal membranes, Solexa sequencing

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Introduction

Retained fetal membranes in cows are an anomaly and are normally released within 12 hours after calving. Approximately 5-40 per cent of dairy cows are affected by retained fetal membranes (Kossaibati and Esslemont, 1997). Retained fetal membranes are a major risk factor for the occurrence of the metritis/endometritis disease complex in early lactation and cost the United Kingdom dairy industry approximately £16 million annually in lost production; not only in UK, but also in other countries with advanced dairy industry. Veterinary clinicians have described the clinical signs, treatment and some associated risk factors of the condition since early Victorian times, and these have not changed over time (Kossaibati and Esslemont, 1997; McNaughton and Murray, 2009).

The first miRNA was found in *Caenorhabditis elegans*, which aberrant expression of lin-4 caused abnormal cell division and proliferation (Lee et al., 1993). Since then, the number of known miRNAs has rapidly increased in recent years (Coutinho et al., 2007), with potentially many more yet to be discovered. MiRNAs are a class of small (18-24 nucleotides), endogenous and non-coding RNAs that bind to target mRNAs and regulate protein expression, either by repressing translation, promoting degradation of the target mRNA (Bartel, 2004; Carrington and Ambros, 2003), or enhancing translation at the posttranscriptional level through the RNA-induced silencing complex (Vasudevan et al., 2007). MiRNA biological roles are diverse and can affect developmental timing, including embryogenesis, organ differentiation, organogenesis, growth and programmed cell death as well as stem and germ line cell maintenance, disease and evolution (Carrington and Ambros, 2003). The key to understanding miRNA regulatory mechanisms is the ability to identify their regulatory targets. The use of computational prediction methods has developed into an important approach for identifying these regulatory targets (Bartel, 2009). It was reported that miRNAs from placenta was related to preeclampsia and placental function (Sadovsky et al., 2015; Xu et al., 2014).

It is known that the maternal placenta and the fetal placenta are fused when fetal membranes are retained in cows. The maternal placenta has the most direct contact with the fetal placenta. However, it is unknown what role miRNAs may play in the maternal placenta in this process. The aim of the present study was to identify novel miRNAs or differentially expressed miRNAs that affect the retention of fetal membranes in the maternal placenta in Holstein cattle. These results will help provide better understanding of the role of miRNAs in retained fetal membrane processes and the mechanisms of target gene expression and regulation.

Materials and Methods

Control group and experimental animals: Experiments were performed following protocols approved by the Institutional Animal Care and Use Committee (IACUC). Six healthy Holstein dairy cows and six Holstein dairy cows with retained fetal membranes of 5-6 years old, 3-4 fetal times, 570 ± 30 kg weight and 30 ± 3.5 kg milk yield were chosen from an experiment cattle farm at Jilin Agricultural University and divided into two groups. The control group (NC) exhibited normal release of the fetal membranes within 12 h after calving while the experimental group (RC) failed to release fetal membranes within 12 h after calving (Clancy et al., 2007).

Sample collection and RNA extraction: For the normal control group (NC), the maternal placentas were collected immediately after normal release of the fetal membrane. For the experimental group (RC), the maternal placentas were collected with a sterile endometrial sampler 12 h after calving. After collection, the maternal placentas were washed three times with a sterile saline solution. Then, the samples were immediately frozen in liquid nitrogen and stored separately at -80℃. Total RNA was extracted from the samples with Trizol (Invitrogen, USA) according to the manufacturer's instructions. Quantity and integrity of the RNA was analysed by Agilent 2100 (Agilent, USA).

Solexa sequencing: Overall flow of the sequencing and bioinformatics analysis for small RNAs was finished by BGI, China. The main steps were as follows: after polyacrylamide gel electrophoresis to separate RNA segments by size, a 18-30 nt strip was excised and a pair of Solexa adaptors was ligated to their 3'and 5'ends. To obtain cDNA, RT-PCR was performed to amplify small RNAs. The cDNA was used for cluster generation and sequencing analysis using the Illumina's Solexa Sequencer according to the manufacturer's instructions. Finally, data processing, size distribution and bioinformatics analysis for the small RNAs was finished by BGI, China, through the software (SOAP, tag2miRNA, tag2repeat, overlap and so on).

Novel miRNA: Prediction software: Mireap (http://sourceforge.net/projects/mireap/) was used to predict novel miRNAs by exploring the secondary structure, Dicer cleavage sites and minimum free energy of unannotated small RNA tags. The RNA tags could be mapped to the genome by characteristic hairpin structures of miRNA precursors, which can be used to predict novel miRNAs (Zhang et al., 2006). New miRNA genes that are paralogs or that are orthologs to known miRNAs were identified by miRAlign (Wang et al., 2005). Other candidates were analysed by MiPred to remove pseudo-pre-miRNAs that had a minimum free energy >-20 kcal/mol or a *P*-value > 0.05 (Jiang et al., 2007). For novel miRNAs, candidates were aligned with the most similar miRNA sequences in the miRbase (Release19 .0).

Differential expression analysis of miRNAs: To identify differential miRNA expression between the two libraries (the normal control group and the experimental group), the expressions of miRNAs in two samples were normalized to obtain the expression of transcripts per million. If the normalized expression value of a given miRNA was zero, its expression value was modified to be 0.01. If the normalized expression (NE) of a given miRNA was less than 1 in both

libraries, the miRNA was removed from future differential expression analysis. The fold-change and *P*-value were calculated from the normalized expression using the following formulas: Normalized expression = (Actual miRNA sequencing read count / Total clean reads count) ×1,000,000.

Fold change = Log2 (experimental group -NE / normal control group -NE)

P-value:

$$P(x|y) = (\frac{N_2}{N_1})\frac{(x+y)!}{x!\,y!(1+\frac{N_2}{N_1})^{(x+y+1)}} \qquad C(y \le y_{\min}|x) = \sum_{y=0}^{y \le y_{\min}} p(y|x)$$

$$D(y \ge y_{\max}|x) = \sum_{y \ge y_{\max}}^{\infty} p(y|x)$$

N1 and x represent the total count of clean reads and the normalized expression level of a given miRNA in NC library, respectively. N2 and y represent the total count of clean reads and the normalized expression level of a given miRNA in RC library, respectively.

Real-time quantitative PCR (Q-PCR): Total RNA was extracted in the maternal placenta from NC (n=6) and RC (n=6) by Trizol according to the manufacturer's instructions. The quantity and integrity of the RNA was analysed by Agilent 2100. U6 was used as an endogenous control. All of the primers were designed according to Chen (Chen et al., 2005). RT was performed using a PrimeScript™ RT reagent Kit (TaKaRa) according to the manufacturer's protocol. An FQD-48A fluorescence quantitative PCR detection system (Applied Bori of China Hangzhou) was used to perform the quantitative analysis using a SYBR Green PCR master mix (Applied TaKaRa). The PCR reaction cycle was similar to previous reports (Ro et al., 2006): 95˚C 4 min, 94˚C 30 sec, 60˚C 30 sec, 72˚C 30 sec, 72˚C 6 min, 35 cycles. Relative miRNA expression levels were calculated by the 2 (-Delta C (T)) methods for relative real-time PCR (Livak and Schmittgen, 2001).

Results

Construction of two small RNA libraries by Solexa sequencing: To identify novel and differentially expressed miRNAs in the normal control group (NC) and the experimental group (RC), two small RNA libraries were constructed by Solexa sequencing. Solexa sequencing provided a total of 6,000,000 and 5,478,059 reads of 3 to 30 nt from the normal control group and experimental group libraries, respectively. After removing low quality, adaptor, or insufficiently tagged sequences, a total of 5,930,853 and 5,367,946 reads of 18 to 30 nt were obtained in the control and experimental groups, respectively. The results are shown Table 1.

Table 1 NC and RC samples of data by Solexa sequencing

Fragment of tape	Quantity		Percent (%)	
	NC	RC	NC	RC
total_reads	6000000	5478059		
high_quality	5962381	5446898	100%	100%
3'adapter_null	2199	1759	0.04%	0.03%
insert_null	1273	6659	0.02%	0.12%
5'adapter_contaminants	21224	36197	0.36%	0.66%
smaller_than_18nt	6806	34304	0.11%	0.63%
polyA	26	33	0.00%	0.00%
clean_reads	5930853	5367946	99.47%	98.55%

In both libraries, the length of the majority of small RNAs was 19 nt-24 nt, which is typical of a Dicer-processed small RNA product. The size distributions (10 nt-30 nt) of the small RNA from the NC and RC samples were similar. For example, in the NC and RC, the 22 nt sequences were the similar small RNAs and accounted for 56.21% and 53.96% of the total sequences, respectively.

In the NC and RC libraries, the total unique reads of common and tissue-specific small RNA. The unique NC-specific sequences accounted for 42.31% of all sequencing reads and for 43.36% in the RC library. The percentages of the NC-specific and RC-specific sequences were 1.78% and 1.74% of the total small RNAs in the two libraries, respectively.

All of the sequencing reads were aligned against the bovine genome (Btau_6.0) using the SOAP Program. It was found that 4,596,223 reads in the NC library matched the bovine genome (5,930,853 reads in total), and a total of 4,270,190 reads matched the RC library (5,367,946 reads in total). There were 93,116 unique reads in the NC library (219,720 unique reads in total) and 98,379 unique reads in the RC library (223,768 unique reads in total), as shown in Table 2. The mature miRNAs were highly enriched in our small RNA library because known miRNAs accounted for 4,254,515 (71.74%) of all sequencing reads in the NC library and 3,920,437 (73.03%) of all sequencing reads in the RC library. Moreover, analysis of the data revealed that the unique reads from known miRNAs presented only a very small fraction of the total number (1.69% and 1.64% in the NC and RC libraries, respectively), as shown in Fig. 1A and Fig. 1B.

Identification of conserved bovine miRNAs: To identify conserved miRNAs in our database, sRNAs that were 18-30 nucleotides long were matched to known bovine mature/precursor miRNA sequences in the miRBase database. It was found that 3,703 unique sequences (4,254,515 reads) were annotated as miRNA candidates in the NC library as well as 3,665 sequences (3,920,437 reads) in the RC library (Figs. 1 A/B). There were 460 and 457 categories of miRNAs (miRNA, miRNA*, miRNA-5p and miRNA-3p) from 486 and 482 independent genomic loci, respectively, from miRBase in the NC and RC libraries, respectively (Table 3).

Table 2 Mapping statistics of NC and RC samples

Gene	Category	Unique sRNAs	Percent (%)	Total sRNAs	Percent (%)
NC	Total sRNAs	219720	100%	5930853	100%
	Mapping to genome	93116	42.38%	4596223	77.50%
RC	Total sRNAs	223768	100%	5367946	100%
	Mapping to genome	98379	43.96%	4270190	79.55%

Table 3 Matching sRNAs to the known miRNA in miRBase

	miRNA	miRNA*	miRNA-5p	miRNA-3p	Precursor miRNA	Matched miRNA precursor unique sRNA	Matched miRNA precursor total sRNA
Known miRNA in miRBase	620	0	82	81	798	-	-
NC	357	0	48	55	486	3764	4256082
RC	357	0	49	51	482	3724	3921111

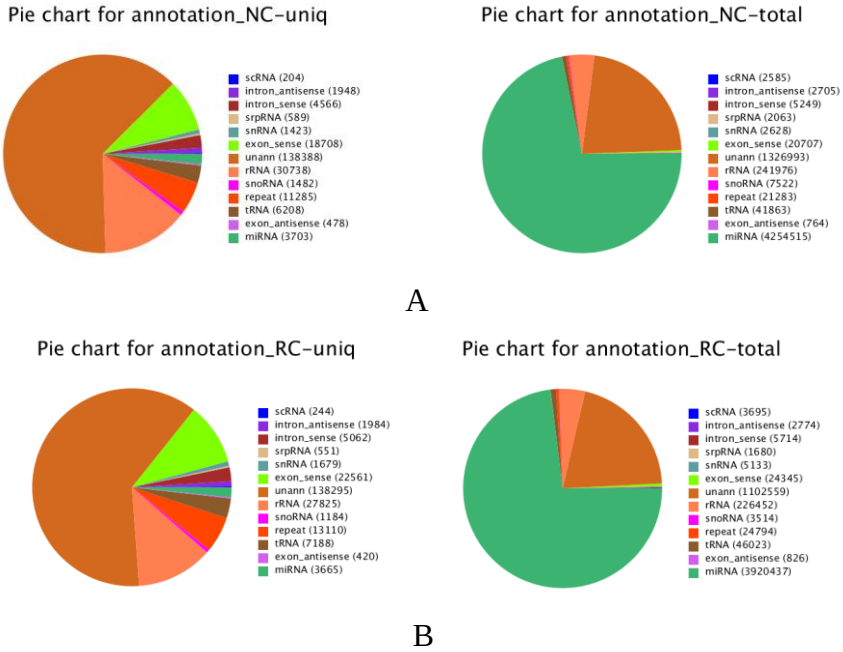

Figure 1 Distribution of small RNA in NC (A) and RC (B)

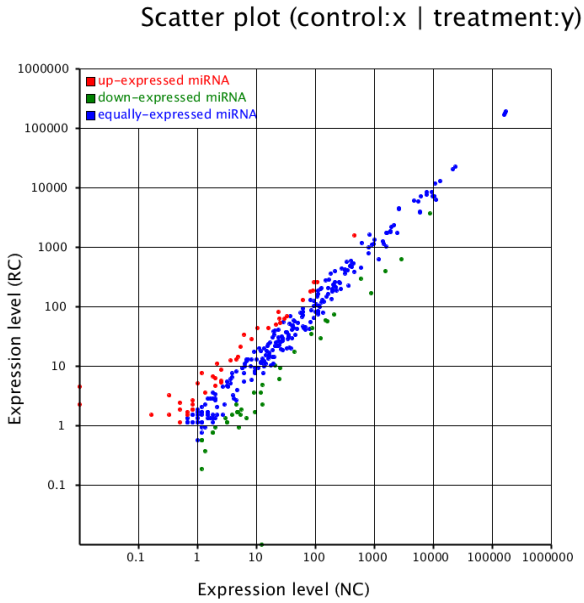

Figure 2 Differential expression of bovine conserved miRNAs between NC and RC tissues is shown. Notes: The X and Y axes show expression levels of miRNAs in the two samples, respectively. The red points represent miRNA with a ratio > 2. The blue points represent miRNAs with ½ < ratio < 2. The green points represent miRNAs with ratio < ½. Ratio = normalized expression of the treatment / normalized expression of the control.

Expression of miRNAs in NC and RC: To estimate whether there was differential expression between the two samples, known miRNAs were counted (for method see Differential expression analysis of miRNAs), and the results are shown in Fig. 2 and list 1. Sixty-nine significantly differentially expressed known miRNAs in the two samples (P<0.05) were discovered, in which 33 RC were up-regulated and 36 were down-regulated compared to NC expression. It is worth noting that bta-miR-423-5p, bta-miR-181a, and bta-miR-185 were down-regulated in RC, and bta-miR-411a, bta-miR-31, bta-miR-424-5p were up-regulated in RC. Not only were they significantly differentially expressed but after standardizing the expression of the samples (sample-std), they were still abundantly expressed. This suggests that differentially expressed miRNAs are related to fetal membrane retention in cows. Even though there were some abundantly expressed miRNAs, others such as bta-miR-1839 and bta-miR-191 had similar expression.

Validation of miRNAs expression with Q-PCR: To validate the presence of known bovine miRNAs, qPCR (Chen et al., 2005) analysis of miRNAs expression was performed in the two samples. In this study, 8 significantly highly expressed miRNAs were chosen to compare between the NC and RC bta-miR-31, bta-miR-424-5p, bta-miR-1839 and bta-miR-1. The qPCR results were compared for deep sequencing, which revealed similar trends for all of the selected miRNAs (Fig. 3).

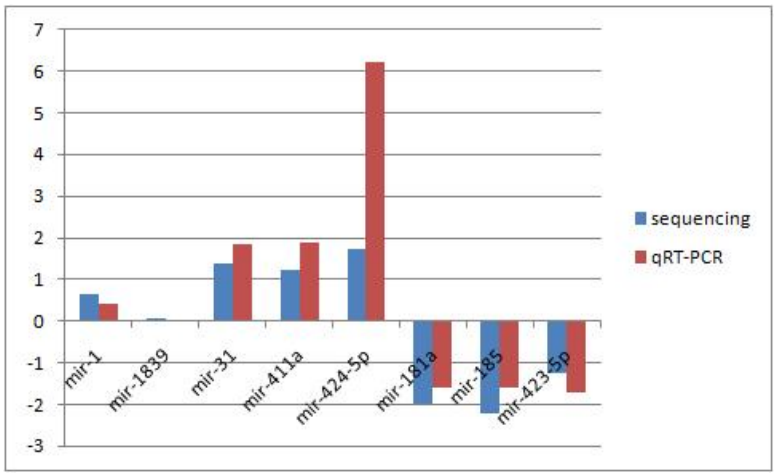

Figure 3 Q-PCR validation of the identified miRNAs using Solexa sequencing technology

Identification of novel cattle miRNAs: In the present study, we identified 36 and 33 novel bovine miRNAs in the NC and RC libraries, respectively. In addition, an examination of pre-miRNAs and other RNAs (tRNA, rRNA, and mRNA) revealed that the miRNAs were significantly different from other RNAs (Zhang et al., 2006). Specifically, more than 90% of the miRNA precursors have a minimal folding free energy index (MFEI) greater than 0.85, which is significantly higher than tRNAs (0.64), rRNAs (0.59), or mRNAs (0.65). The results indicate that the MFEI can easily be used to distinguish miRNA from other non-coding and coding RNAs (Sun et al., 2014). This criterion can be used to predict novel bovine miRNAs, and in our two libraries (24) pre-miRNAs had a MFEI greater than 0.85.

Prediction of target genes and bioinformatics analysis: MIREAP software was used to predict target genes of known miRNAs. There were a total of 999,095 and 983,378 putative target sites in the known NC and RC miRNAs, respectively. There were a total of 264,973 target sites in the differentially known candidates of the differentially expressed miRNAs (diff-miRNAs) for Gene Ontology (GO) categories and KEGG functional annotations. GO revealed that in the NC library, small molecule binding was significant enrichment (P<0.05) (Table 4); nucleoside binding, purine nucleoside binding, ribonucleoside binding, purine ribonucleoside binding were significant enrichment (P<0.05) in the RC library (Table 4). KEGG pathway displayed that Endocytosis and Epstein-Barr virus infection were significant enrichment (P<0.05) in the NC library (Table 5); interestingly, Tight junction, Lysosome and Focal adhesion were significant enrichment (P<0.05) in the RC library (Table 5). After analysing all of the target gene candidates of known diff-miRNAs (n=333) in the two libraries, GO result had no significant enrichment (Table 4), however, the KEGG pathway results were related to many important pathways, and interestingly, Regulation of actin cytoskeleton signaling pathway and MAPK signaling pathway were significant enrichment (P<0.05) (Table 5).

Notably, among the miRNAs, three were down-regulated: bta-miR-423-5p, bta-miR-181a, bta-miR-185; and three were up-regulated: bta-miR-411a, bta-miR-31, and bta-miR-424-5p, which were highly expressed and had a significant fold-change. They may be important for fetal membrane retention in cows.

Discussion

Fetal membrane retention in cows is a common postpartum disease that is a serious threat to animal fitness. Animals can develop secondary diseases, such as endometriosis, resulting in large economic losses. Until now, the mechanism of the disease is still unclear and the specific molecular basis is unknown. An important reason for this uncertainty is the fact that disease susceptibility is a multifactorial and complex phenotype that is not the result of any single gene acting in isolation but rather is attributable

to perturbations at the network or system level (Barabasi et al., 2011). It is estimated that approximately 30% of all protein-coding genes are regulated by miRNAs (Lewis et al., 2003). Therefore, to better understand the biological mechanisms between NC and RC, samples of the maternal placenta from healthy and fetal membrane-retaining Holstein dairy cows were collected and two pooled miRNA libraries were constructed by Solexa sequencing.

Of the mappable sequences, the majority of the small RNAs were 21-24 nt in size and correlated with the known specificity of Dicer processing and the features of mature miRNAs (Lau et al., 2001). Our study found that the 22 nt sequences were the most common and that the 21 nt and 23 nt miRNAs were more common than others, regardless of the library. This is in agreement with Chen, who reported that 21-23 nt sequences were significantly enriched over others and that 22 nt miRNA was almost half of the sequences in the back fat of Large White and Meishan pigs (Chen et al., 2012). Our results are consistent with previous studies by Sun et al. (2014), but do not agree with Huang et al. (2011). After further analysis, it is believed that the results of the present study are similar to those of Sun et al. (2014), but different from those of Huang et al. (2011), although all of the results were derivd from cattle.

Table 4 GO functional enrichment of miRNAs potential targets in the samples

Sample	Gene ontology term	Cluster frequency	Genome frequency of use	*P*-value
NC	Small molecule binding	1613 out of 8740 genes, 18.5%	1623 out of 8871 genes, 18.3%	0.35737
	Ion binding	2325 out of 8740 genes, 26.6%	2347 out of 8871 genes, 26.5%	1
RC	Nucleoside binding	1157 out of 8651 genes, 13.4%	1167 out of 8871 genes, 13.2%	0.01294
	Purine nucleoside binding	1151 out of 8651 genes, 13.3%	1161 out of 8871 genes, 13.1%	0.01454
	Ribonucleoside binding	1151 out of 8651 genes, 13.3%	1161 out of 8871 genes, 13.1%	0.01454
	Purine ribonucleoside binding	1151 out of 8651 genes, 13.3%	1161 out of 8871 genes, 13.1%	0.01454
Differential expression miRNAs	Small molecule binding	1603 out of 8651 genes, 18.5%	1623 out of 8871 genes, 18.3%	0.07568
	Protein binding	1570 out of 8850 genes, 17.7%	1571 out of 8871 genes, 17.7%	1
	Heterocyclic compound binding	2797 out of 8850 genes, 31.6%	2802 out of 8871 genes, 31.6%	1
	Metal ion binding	1570 out of 8850 genes, 17.7%	1571 out of 8871 genes, 17.7%	1
	Catalytic activity	3686 out of 8850 genes, 41.6%	3692 out of 8871 genes, 41.6%	1
	Hydrolase activity	1457 out of 8850 genes, 16.5%	1459 out of 8871 genes, 16.4%	1

Table 5 KEGG pathway enrichment of miRNAs potential targets in the samples

Sample	Pathway	Target genes with pathway annotation (10473)	All genes of the species with pathway annotation (10623)	*P*-value	Pathway ID
NC	Endocytosis	292 (2.79%)	292 (2.75%)	0.01483337	ko04144
	Epstein-Barr virus infection	244 (2.33%)	244 (2.3%)	0.02988176	ko05169
RC	Dilated cardiomyopathy	187 (1.79%)	187 (1.76%)	0.06835474	ko05414
	Tight junction	265 (2.55%)	266 (2.5%)	0.01355169	ko04530
	Lysosome	153 (1.47%)	153 (1.44%)	0.02702647	ko04142
	Focal adhesion	283 (2.73%)	285 (2.68%)	0.03661853	ko04510
	ECM-receptor interaction	124 (1.19%)	124 (1.17%)	0.05380286	ko04512
	Protein digestion and absorption	120 (1.16%)	120 (1.13%)	0.0591539	ko04974
Differential expression miRNAs	Pathways in cancer	373 (3.52%)	373 (3.51%)	0.4236714	ko05200
	Regulation of actin cytoskeleton	337 (3.18%)	337 (3.17%)	0.4609076	ko04810
	MAPK signaling pathway	322 (3.04%)	322 (3.03%)	0.4773311	ko04010
	HTLV-I infection	297 (2.8%)	297 (2.8%)	0.5059569	ko05166

In this study, it was found that miRNAs accounted for a significant proportion (73.03% and 71.74%) of RC and NC. Most of the six miRNAs (down-regulated: bta-miR-423-5p, bta-miR-181a, bta-miR-185; up-regulated: bta-miR-411a, bta-miR-31, bta-miR-424-5p) were highly expressed and had a significant fold-change. It has been reported that miRNAs are often clustered and sometimes co-expressed from the same primary transcript, leading to the hypothesis that they may share functional relationships (Bartel, 2004). This means that diff-miRNAs are likely to influence bovine fetal membrane retention in some way. The six miRNAs are likely to play a part in regulating this process.

Some authors (Scott et al., 1982; Shixin et al., 2011) have suggested that uterine fatigue and inadequate uterine contractions are the primary factors involved in placental retention. However, in our

opinion, uterine fatigue and inadequate uterine contractions of the cows also depend on the energy the organism can supply and the muscular strength of the uterus. Based on this viewpoint, two aspects of a possible theory about bovine fetal membrane retention are suggested.

The first is the biological cause of fetal membrane retention in cows. Inflammation and adhesion occur in joint portions of the maternal and fetal placenta (Benedictus et al., 2015). Inflammation is mainly an immune response. The report noted that bovine fetal membrane retention was likely connected to the immune response (Rapacz-Leonard et al., 2014). An additional link that exists between metabolism and inflammation and that is currently under intensive investigation is the role of NAD+, sirtuins (SIRTs), and AMP-dependent protein kinase (AMPK) in suppressing inflammation (McNaughton and Murray, 2009). NAD+ and AMPK interfere with the energy supply. If the energy supply decreases, uterine fatigue and inadequate bovine uterine contractions will likely increase. Meanwhile, several previous *in vitro* studies strongly suggest the roles for miRNAs in regulating bovine immunity (Dilda et al., 2012). MiRNAs are likely to play a key role in regulating the links between inflammation and metabolism (Lawless et al., 2014). For example, human SIRT1, which limits inflammation as discussed above, has been shown to be a target of miR-34a (Yamakuchi et al., 2008), and miR-451 has also been shown to regulate the expression of several pro-inflammatory cytokines in mice in response to influenza infection (Rosenberger et al., 2012). In this study, there are some target gene candidates of diff-miRNAs that are related to inflammation, such as bta-miR-423-5p. The software analysis indicated that it might be the target gene of aldose reductase (AKR1B1), which is related to immunity. Therefore, it is proposed that diff-miRNAs probably regulate the expression of target genes that mediate inflammatory reactions to reduce the organism's energy output, resulting in uterine fatigue and inadequate uterine contractions, followed by fetal membrane retention.

Second, a report also noted that heart muscle is activated by Ca2+ to generate extension and contraction, and the signalling pathway involves allosteric mechanisms in the thin filament (Sevrieva et al., 2014). Previous results identify the promotion of actin-based cell-cell adhesion as a newly described mechanism of action (Fenton et al., 2015).

In the present study of known diff-miRNAs GO and KEGG, there were many functions and pathways related to the Ca2+ and actin, including actin cytoskeleton signalling pathway and MAPK signaling pathway which were significant enrichment. They were closely related to muscle contraction. Moreover, comparing the RC library to the NC library, Tight junction and Focal adhesion were significant enrichment. More importantly, they were closely related to cell adhesion, but it happened where the maternal placenta of Holstein cattle with retained fetal membranes. Therefore, we strongly believe that miRNAs that are differentially expressed before and after bovine fetal membrane retention lead to a maternal placenta change, increasing the chance of fetal membrane retention. Granted, this is a hypothesis, and a validation of this finding is underway.

MiRNAs are abundantly and stably expressed in a range of accessible tissues, including serum, milk, urine, saliva, and semen, where they can be readily measured (Chen et al., 2010). In 2009, Prometheus Laboratories released a miRNA biomarker to accurately identify 25 different tumour types (Ajit, 2012). As a potential biomarker, miRNAs have a high information content, and the expression profiles of small numbers of them have been used as a diagnostic tool for disease (De Guire et al., 2013). Perhaps, a more convenient, accurate and inexpensive biomarker will be developed to take advantage of these miRNAs features in the future.

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บทคัดย่อ

Solexa Sequencing ในการตรวจหาและตรวจแยกการแสดงออกของ microRNAs ในรกของโคนมที่มีภาวะเยื่อบุรกค้าง

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MicroRNAs (miRNAs) มีบทบาทในควบคุมการแสดงออกของยีน และมีความสำคัญในการพัฒนาและการทำงานของเซลล์และอวัยวะในโค การศึกษาครั้งนี้ ตรวจหา miRNAs ชนิดใหม่ และ ตรวจแยกการแสดงออกของ miRNAs ในรกของโคนม Holstein ที่มีภาวะเยื่อบุรกค้าง (กลุ่มทดลอและกลุ่มควบคุม กลุ่มละ 6 ตัว) การศึกษาพบว่า มีสายรหัสพันธุกรรม 4,596,223 สาย ที่ตรงกับ โครโมโซมของรกแม่โคปกติ (5,930,853) และพบว่า มีสายรหัสพันธุกรรม 4,270,190 สาย ที่ตรงกับรกของแม่โคที่มีภาวะเยื่อบุรกค้าง (5,367,946) ในการศึกษาครั้งนี้ สามารถระบุ miRNA ชนิดใหม่ จำนวน 36 ชนิด (ในเยื่อบุรก ทั้งในโคปกติและในโคที่มีภาวะเยื่อบุรกค้าง) และ จำนวน 33 ชนิด (ในรกแม่โค) โดยพบว่า miRNAs ทั้ง 69 ชนิดมีความแตกต่างกันอย่างมีนัยสำคัญระหว่างโคทั้งสองกลุ่ม ($P < 0.05$) โดยมี miRNAs จำนวน 33 ชนิดที่มีการแสดงออกแบบเพิ่มขึ้น และ 36 ชนิด ที่มีการแสดงออกแบบลดลง และสามารถตรวจยืนยันการแสดงออกของ miRNAs ด้วยวิธี real-time PCR เป็นที่น่าสนใจว่ามี miRNAs จำนวน 6 ชนิดที่มีการแสดงออกแตกต่างกันอย่างมาก และมีการเปลี่ยนแปลงมากกว่า 1.2 เท่า (การแสดงออกลดลง: bta-miR-423-5p, bta-miR-181a, bta-miR-185; การแสดงออกเพิ่มขึ้น: bta-miR-411a, bta-miR-31, bta-miR-424-5p) โดยสรุป miRNAs เหล่านี้อาจมีส่วนสำคัญ ในการเกิดภาวะเยื่อบุรกค้างในโค ซึ่งการหาสาเหตุ ในการเกิดภาวะเยื่อบุรกค้างในโค สามารถอธิบายได้ด้วยการวิเคราะห์ bioinformatic ของการแสดงออกของ miRNAs

คำสำคัญ: รก miRNA เยื่อบุรกค้าง Solexa sequencing

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