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Original Article

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Abstract

Retained foetal membrane (RFM) is a common reproductive pathology that harms the health of dairy cows. The aim of this study was to clarify the pathogenesis of RFM and screen for an early clinical diagnostic marker of this disease by using comparative proteomics and bioinformatics. Three RFM placental tissues from three cows and an equal number of normal placental tissues were collected for this study. There were 240 spots showing significant differential expressions of known proteins between the two types of foetal placental tissue samples. Between the two maternal placental tissue samples, there were 214 spots with differentially expressed levels of known proteins. Ten spots were successfully picked from the gels of the two foetal placental tissues. Of these, only seven showed significant differences in protein expression levels (BSA, alpha enolase, apolipoprotein A-I, annexin A8-like 1, serine proteinase inhibitor, glutathione transferase, and transketolase). Nine distinct spots were successfully chosen from the two maternal placental tissues. Of these, only five spots showed significant differences in protein expression (aldose reductase, heat shock 27 kDa protein 1, serine (or cysteine) proteinase inhibitor, alpha enolase, and annexin A2). The cause and influencing factors of this disease are related to fibrinolysis, pyruvate metabolism, inflammatory response and oxidative stress.

Keywords: dairy cow, retained foetal membranes, proteomics, clinical diagnostic marker

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Introduction

Retained foetal membrane (RFM) is defined as a placenta that cannot be discharged normally from the uterus and birth canal within 12 h. The morbidity of RFM can reach up to 60% during the summer. Because RFM affects herd health, milk production and reproductive efficiency, this condition is economically one of the most important disturbances during the postpartum period in cattle (Kamemori et al., 2011; Beagley et al., 2010; Rapacz et al., 2012).

During bovine RFM, the morbidity of mastitis increases 6- to 8-fold, and the morbidity of laminitis increases 5- to 7-fold. RFM can also cause septicaemia, and over 72% of RFM cases result in endometritis. In this case, endometritis can affect the involution of the uterus, leading to infertility. Therefore, the time interval that cows spend in calving must be prolonged and infertile cows must be forcibly weeded out ahead of schedule (Sprekeler et al., 2012; Kimura et al., 2003; Goff, 2008; Seifi et al., 2007).

As the scale of cattle rearing has continuously increased and the use of highly intensive breeding has become more frequent, factors affecting RFM have become more complex (Beagley et al., 2010; Kumari et al., 2014; Peter, 2013). Domestic and international reports indicate that the morbidity of RFM ranges from 5% to 50%. In England, RFM has reportedly caused a loss of nearly $16 million. These losses caused by RMF are greater in China due to the uneven breeding levels.

As the pathogenesis of RFM is complex (Provencher et al., 1988), there are abundant factors that influence this condition. Identifying the undefined pathogenic factors that give rise to RFM will affect the efficiency with which RFM can be controlled. Almost all previous studies of RFM were confined to the analysis of in vivo and in vitro indicators of the condition, which cannot objectively and comprehensively reveal key issues about the aetiology of RFM.

Therefore, the aims of this study were to filter and analyse the differentially expressed proteins between the physiological and pathological states, use mass spectrometry to identify these proteins and analyse their relevant bioinformatics data, identify a key protein involved in the disease development, and provide a basis for exploring factors that induce RFM. Significantly, these approaches may be used to illuminate the pathogenesis of RFM, to identify early clinical diagnostic markers of the disease, and to screen medicines with the potential to treat RFM.

Materials and Methods

Sample collection: Three RFM placental tissues from three cows and an equal number of normal placental tissues were collected for this study. Cows that were similar in age, weight, and amount of lactation were selected from an intensive and standardized dairy farm in Heilongjiang province. RFM placentas were selected from cows that did not vaginally deliver the placenta within 12 hours, whereas normal placentas were collected from cows that vaginally discharged the placental tissues within 12 hours and showed no clinical symptoms.

After the placentas were collected, the maternal placental tissue was obtained from the RFM and normal placentas using an endometrial sampler. The samples collected from the placentas were washed in cold normal saline, subpackaged, and cryopreserved with liquid nitrogen before storage at -80℃.

Protein extraction: Total protein was extracted from the placental tissues using ReadyPrep Total Protein Extraction Kit (Bio-Rad Laboratories Inc., Hercules, California) as the specifications described (Cilia et al., 2009; Tan et al., 2011; Wolff et al., 2011). All protein samples were stored at -80℃ until subsequent analyses.

2D-PAGE: The first dimension of 2D-PAGE is the isoelectric focusing, in which the proteins move at different rates to a certain location according to their respective pI values. In the process of isoelectric focusing, a protein can move to its constant loci from each direction. To minimize the variation in the second dimension, the normal and RFM protein extracts were run and stained at the same time in a twin gel electrophoresis system (Gharesi-Fard et al., 2010; Jin et al., 2008). The protein samples were directly extracted from the placental tissues using hydration fluid (7 mol/L urea, 40 g/L CHAPS, 30 mmol/L Tris, and 2 mol/L thiourea). The protein samples were diluted in IPG buffer (1% DDT, 40% ampholyte, and bromophenol blue). Protean IEF Cell was used (IPGPhor, Amersham Biosciences) for the first dimension of the 2D-PAGE, which was performed using a voltage increase with the five following steps: hydration at 50 V for 13 h; desalination at a rapid gradient to 4000 V in 2 h; a linear gradient to 10,000 V in 3 h; a rapid gradient to 10,000 V over 6 h; and a steady state at 500 V for 6 h. After the first dimension, the proteins were separated in the second dimension using 12% Tris-glycine gels 2DE gels in equilibrium buffer I (1% DTT, 30% glycerol, 2% SDS, 0.002 % bromophenol blue, 50 mM Tris-HCl pH 8.8, 6 M urea) for 15 minutes. The proteins were then separated in equilibrium buffer II (50 mM Tris-HCl pH 8.8, 6 M urea, 2.5% iodoacetamide). IPG strips were loaded onto 10% SDS-polyacrylamide gels and sealed with 1% agarose solution. Electrophoresis was performed in EttanDALT II (Bio-Rad Laboratories, USA) at 20℃ supplying constant power in two steps: 60 V for 30 min and 80 V for 16 h.

Dyeing, spot excision and trypsin digestion: The RFM and normal 2D gels were dyed with silver nitrate. The gels were placed in stain-fixative for 3 hours and then were washed three times with deionized water every 10 minutes. The stain-fixative was replaced with sensitizing solution for 30 minutes. The gels were then washed three times with deionized water every ten minutes. Silvering solution was used to silver stain the gels for 20 minutes, and then the gels were washed with deionized water. Colour-substrate solution was added to colour the gel and chemical reaction was stopped using stop solution for 10 minutes. The gel was washed with water and stored in deionized water. The stained gels were scanned and images were analysed using the ImageMaster 6.0 program (General

Electric Healthcare, Uppsala, Sweden). Parameters of protein spot detection were set as follows: minimal area=10pixels; smooth factor=2.0; and saliency=100.0. Two identical protein spots from the the RFM and normal groups that had 1.5-fold difference were considered to be spots of differentially expressed proteins (Ma et al., 2013; Parra-Torres et al., 2014; Kankofer et al., 2014). The spots were processed after excision (Parra-Torres et al., 2014). The spots were first washed three times with deionized water and then were washed twice with 100 μL of 25 mM (NH4)2CO3/50% CAN for 10 minutes per wash. Peptides were digested using 20 μL trypsin at a concentration of 12.5 ng/μL at 4℃ for 30 minutes. Then, 25 μL of 25 mM NH4HCO3 in trypsin was added. This chemical reaction was sustained for 8 hours at 37℃. The resulting peptides were extracted with TFA solution and centrifuged.

Statistical analysis: The resulting peptide extracts were analysed by SELDI-TOF-MS using a reflection type scanning mode in the range of 700 to 4000 Da. The laser energy for mass spectrometry was 5000, whereas the energy for the secondary mass spectrometry was 5500.

The primary mass spectrum data and the secondary mass spectrum data were obtained after mass spectrometry. The primary and secondary mass spectrum data of each spot were integrated and searched for in NCBI.

Relative-quantitative real-time polymerase chain reaction (QRT-PCR): Total RNA was extracted from three RFM placental tissues from three cows and an equal number of normal placental tissues. Reverse transcription of all RNA samples to first-strand cDNA was performed with PrimeScript™ Transcriptor First Strand cDNA Synthesis Kit Transcriptor (Switzerland, Roche) according to the specification. To identify the differentially expressed protein, real-time PCR was performed by using SYBR Green method (Applied Biosystems, UK) (Switzerland, Roche). Real-time primers used to analyze RFM-related genes was designed by Primer Premier 5.0 (Table 1). β-actin was selected as internal reference. The PCR protocol consisted of a cycle at 95℃ for 1 min followed by 35 cycles consisting of 15 sec at 95℃ and 15 sec at 57℃ as the annealing temperature, and then extending for 15 sec at 72℃ and extending for 5 min at 72℃.

Table 1 PCR primers used to analyze RFM-related genes

Gene	Tm	Product Size	Primer sequences
β-actin	56	403	F : GTCATCACCATCGGCAATGAG R : GCTAACAGTCCGCCTAGAAGCA
Transketolase	56	112	F : CCAGGTGACTGTGATTGGTG R : CTTGATGGTGAAAGGGTCCA
Apolipoprotein A1	55	131	F : CTGACCTTGGCTGTGCTCTT R : CCACTATCCTTGATTGCTTCC
α-enolase	50	261	F : TGGCAAGCACGAGGAAAG R : GGACAGAGAGGAGGAGACCA
Serineproteinase inhibitor	56	108	F : CCCAGTGAAAGCACGGACT R : AAACACCTCCACCTCCTCCT
Glutathione transferase	57	113	F : GAGAACAAAGAAGCCCTGGA R : TTGCCCGACCTGTAGAACTC
Heat shock protein 27	58	144	F : GCTTCACTCGCAAATACACG R : AGGTGACGGGAATGGTGAT
Apolipoprotein A8	55	113	F : CAAGAGCATTGAGGACAGCA R : CAAAGTAGCCGTGGAGGTTC
Apolipoprotein A2	57	123	F : TCTGAACATTGAAACAGCCATC R : TTGGTCCTTCTCTGGTAGGC
Aldosereductase	57	150	F : GACCAAGATGACTGGGAAGC R : CTGGTTGACTTTCAGCAGGAG
BSA	55	439	F : TCCCAGCAGTGTCCATTT R : AGGCAGGCACCTTTATC

Results

Identification of differentially expressed proteins: Two sets of images were obtained of the foetal placental tissues taken from the bovine placentas used in our experiments. The 2-DE silver staining image of the normal foetal membrane (NFM) foetal placental tissue is presented in Fig. 1A, while the 2-DE silver staining image of the RFM foetal placental tissue is presented in Fig. 1B. The 2-DE silver staining image of the NFM maternal placental tissue is presented in Fig. 1C, while the 2-DE silver staining image of the RFM maternal placental tissue is presented in Fig. 1D.

These four silver staining images were analysed as described. It was found that there were 240 significantly differentially expressed spots corresponding to known proteins in the two samples from foetal placental tissues. Of these, 212 spots consisted of proteins that were up-regulated in the RFM tissues compared to the NFM tissues, while 28 spots represented proteins that were down-regulated in the RFM tissues compared to the NFM tissues. The results of the differential gel electrophoresis for the RFM and NFM foetal placental tissues are presented in Fig. 2A. There were 214 spots in which known proteins were significantly differentially expressed between the two samples of maternal placental tissues. One

hundred and thirty four spots contained proteins that were up-regulated in the RFM tissues compared to the NFM tissues, whereas 80 spots contained proteins that were down-regulated in the RFM tissues compared to the NFM tissues. The results of the differential gel electrophoresis for the RFM and NFM maternal placental tissues are presented in Fig. 2B.

The silver-stained gels with more obvious fold changes at independent points were selected and isolated using artificial digging. Enzymolysis was performed on the points in the proper positions, and after the peptide fragments were extracted from the gels, SELDI-TOF-MS was used for the mass spectrometric analysis. The data obtained from the mass spectrometric analysis were compared with the NCBI database in order to identify the proteins which were differentially expressed in the RFM and NFM placentas.

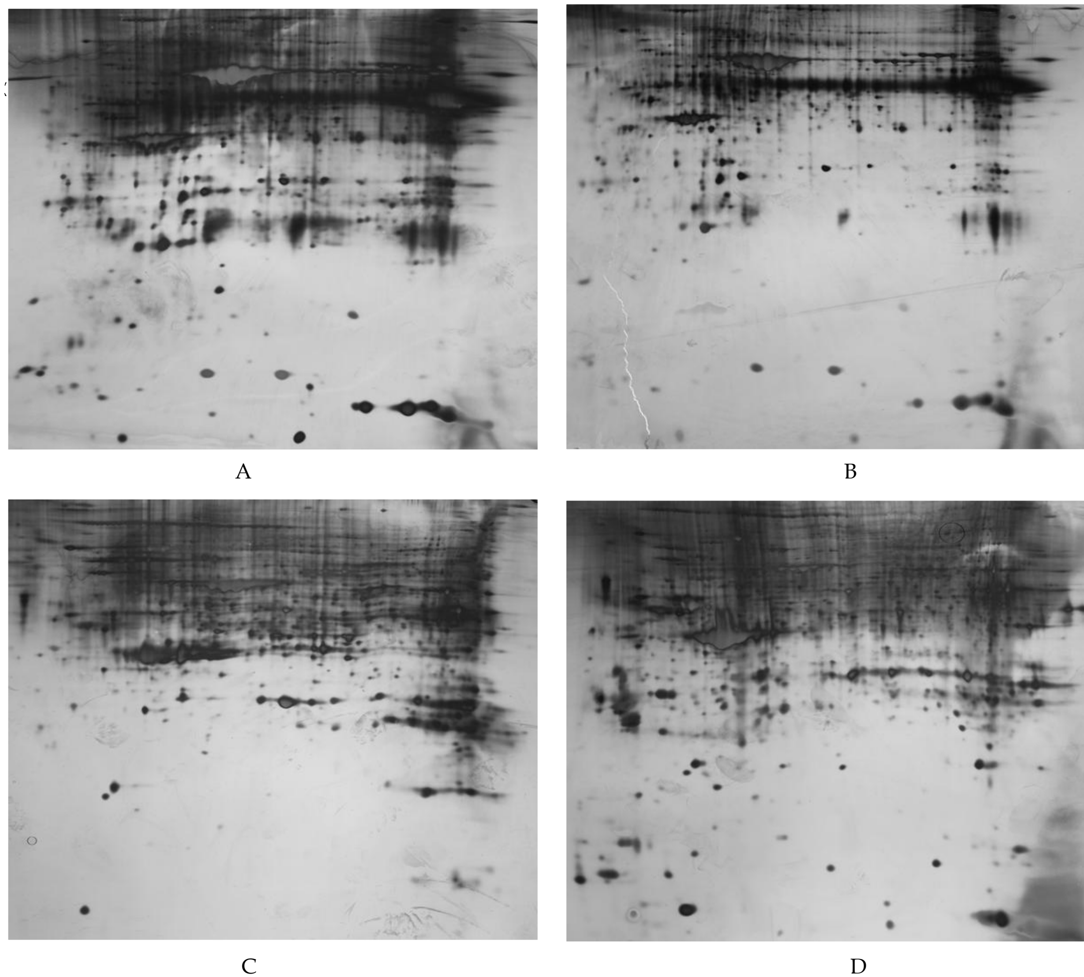

Figure 1 2-DE silver staining image. A: NFM foetal placental tissue. B: RFM foetal placental tissue. C: NFM maternal placental tissue. D: RFM maternal placental tissue.

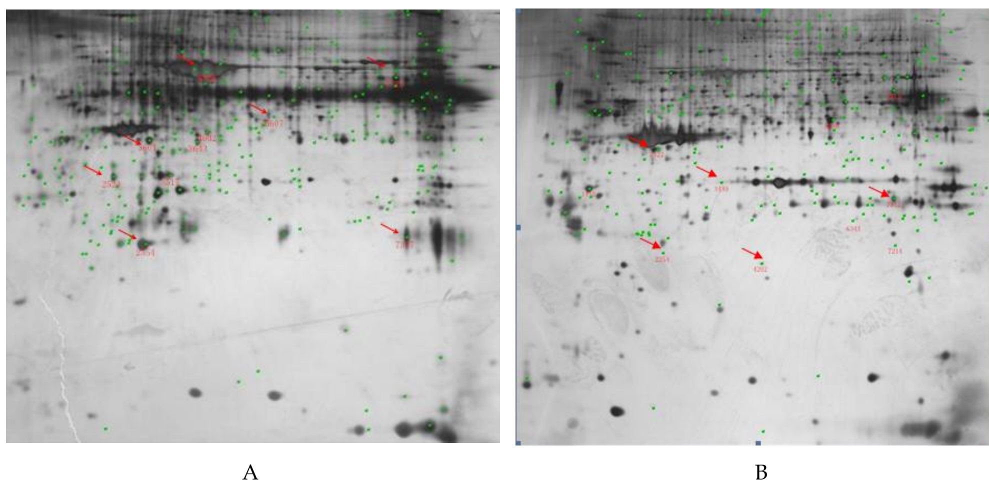

Figure 2 Results of differential gel electrophoresis. A: RFM and NFM foetal placental tissues. B: RFM and NFM maternal placental tissues.

Table 2 Quantities of proteins that were differentially expressed in RFM and NFM foetal placental tissues or maternal placental tissues

Spot no.	RFM	NFM	Ratio
2354	301489.6	1493	0
2524	96130.3	303.1	0
3601	119326.8	3758.1	0.03
4705	1697.9	86101.3	50.71
5607	792.8	113511.8	143.17
7317	202738.6	251.4	0
7715	75997.1	2241.1	0.03
2422	62419.7	5823	0.09
3439	1984.6	69194.4	34.87
4202	9823.3	194191.5	19.77
5536	64404	3033.3	0.05
7312	54168.9	3335.5	0.06

Table 3 MS results of RFM and NFM foetal placental tissues or maternal placental tissues

Spot no.[a]	NCBI no[b].	Protein name[c]	Mr[d]	pI[e]	Expression[f]
2354	gi|245563	apolipoprotein A-I, [Bos=cattle,Friesian-Holstein male calves aged 2-4 weeks, Peptide,247	28414.8	5.57	+
2524	gi|27806317	annexin A8-like 1 [Bos taurus]	36763.6	5.3	+
3601	gi|27807517	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6 [Bos taurus]	42533.2	5.44	+
4705	gi|1351907	: Full=BSA;	69248.4	5.82	_
5607	gi|4927286	alpha enolase [Bos taurus]	47247.3	6.44	_
7317	gi|29135329	glutathione transferase	23598	6.89	+
7715	gi|51491841	transketolase [Bos taurus]	67862.7	7.56	+
2422	gi|27807517	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6 [Bos taurus]	42533.2	5.44	+
3439	gi|162652	aldose reductase (EC 1.1.1.21)	33946.4	5.68	-
4202	gi|61553385	heat shock 27kDa protein 1 [Bos taurus]	17544.9	6.49	-
5536	gi|4927286	alpha enolase [Bos taurus]	47247.3	6.44	+
7312	gi|27807289	annexin A2 [Bos taurus]	38587.8	6.92	+

[a] Spots number in Fig. 6. Spots in the same line were identified as same protein. [b] Protein ID accessed from NCBI database. [c] Spots in the same line were identified as same protein. [d] Theoretical molecular weight in kDa. [e] PI [f] Up- and downregulation of spots in RFM MS foetal and maternal placental tissue as compared with NFM is indicated by + and -, respectively.

The quantities of proteins that were differentially expressed in the RFM and NFM foetal placental tissues are presented in Table 2. Chosen spots are labelled in Fig. 2A. The quantities of proteins that were differentially expressed in the RFM and NFM maternal placental tissues are presented in Table 2. Chosen spots are labelled in Fig. 2B. The result shown in Table 2 was obtained by SELDI-TOF-MS technology.

Twenty differentially expressed spots in the silver-stained gels were identified using the MALDI–TOF-MS, of which 20 mass spectrometric results and 20 peptide mass fingerprinting results were obtained. From the mass fingerprinting results, it was found that all of the signal-to-noise ratios of these peaks were greater than 20:1. The peptide mass numbers were centred in a range of 1000-2000 Da, indicating that the enzymolysis was sufficient.

The NCBI database was searched and the 20 results which were obtained by peptide mass fingerprinting were matched with known proteins (Scored>65, P<0.05); this cut-off identified related proteins down to the level of mustard peptides. Then, the proteins were verified by synthesis

Ten spots were successfully picked from the gels of the RFM and NFM foetal placental tissues. Of these, only seven showed significant differences in the protein expression levels. As shown by the MS analysis, two out of seven differentially expressed proteins were down-regulated (BSA, alpha enolase), while the remaining five spots (apolipoprotein A-I, annexin A8-like 1, serine (or cysteine) proteinase inhibitor, glutathione transferase, and transketolase) showed increased expression in the RFM foetal placental tissues compared to the NFM tissues. The MS

results for the RFM and NFM foetal placental tissues are presented in Table 4.

Nine distinct spots were successfully chosen from the results of the MS analysis of the RFM and NFM maternal placental tissues. Of these, only five spots showed significant differences in protein expression. Two of the five differentially expressed proteins were down-regulated (aldose reductase, heat shock 27 kDa protein 1), while the remaining three spots (serine (or cysteine) proteinase inhibitor, alpha enolase, annexin A2) showed increased expression in the RFM maternal placental tissues compared to the NFM tissues. The results of the MS analysis of the RFM and NFM maternal placental tissues are presented in Table 4.

***Relative-quantitative real-time polymerase chain reaction (QRT-PCR)*:** The result of QRT-PCR analysis is presented in Fig. 3. Fig. 3 shows that the expression of apolipoprotein A-I、serpin、glutathione transferase, apolipoprotein A8 at the RNA level was significantly up-regulated, and transketolase was significantly down-regulated in the RFM foetal placentas as compared with the normal placentas.

The result of QRT-PCR analysis is presented in Fig. 4. Fig. 4 shows that the expression of serine proteinase inhibitor、apolipoprotein A2 at the RNA level was significantly up-regulated, and heat shock protein 27 was significantly down-regulated in the RFM maternal placentas as compared with the normal placentas.

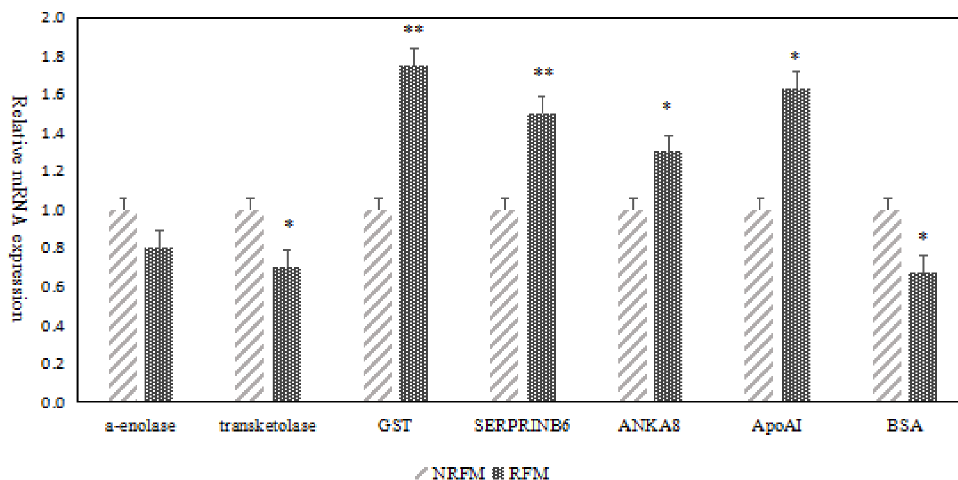

Figure 3 Expression of differential expression protein mRNA in foetal placental tissue

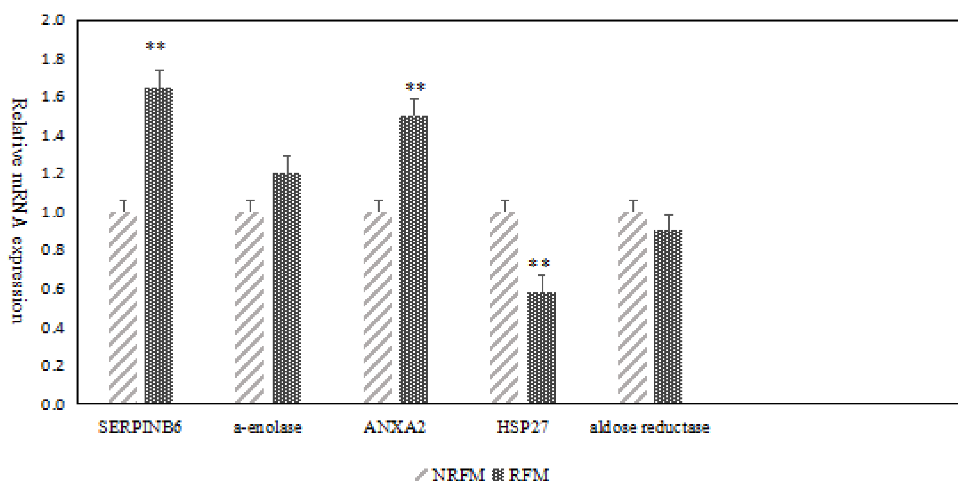

Figure 4 Expression of differential expression protein mRNA in maternal placental tissue

Discussion

Bovine serum albumin (BSA) is a type of globulin synthesized by the cow liver. The content of BSA is abundant in the circulatory system, where it has important physiological functions in maintaining the osmotic pressure of the body, blood circulation, material metabolism, and so on (Prashanth et al., 2013; Saha et al., 2014). BSA can permeate into tissues under physiological conditions and re-access the blood vessels via lymphocinesia (Hossain et al., 2011). Due to the uterine contractions and inflammation which occur during birth, the microcirculatory system of the placental tissue results in the accumulation of BSA in the placental tissues. The protein expression index of BSA changes in the cows with RFM, but the mechanism of this change remains unclear. A past study has indicated that the changes in antioxidant levels and the immune state are related to RFM. When RFM occurred in dairy cows, the ability to scavenge free radicals declined, the activity of GSH-PX reduced (Kankofer et al., 2013; Vaughan et al., 2002), and the MDA

(malonaldehyde) content increased noticeably, suggesting that GSH-PX and MDA are involved in RMF. According to the experimental results described above, the reduction in oxidation resistance in the body results in excess of free radical. The excess of free radical in the body will attract the placental tissue cells, then the foetal placenta will adhere to the maternal placenta to separate the microvilli organization of the foetal placenta apart from the maternal placenta. Glutathione transferase and the 27 kDa heat shock protein are both involved in the oxidation reactions and may affect the development of RFM by participating in the organic oxidation reaction (Dodge et al., 2006; Abdulsid et al., 2014; Wataba et al., 2004). Five proteins (annexin A2, annexin A8, alpha enolase, apolipoprotein A-I, and annexin A5) participate in the fibrinolytic system and play a negative role in the process of blood clotting. Annexin A2, annexin A5 and annexin A8 can bind to cell surface phospholipids in order to competitively prevent these phospholipids from participating in blood clotting. These annexins also have anticoagulant functions under physiological conditions, as they participate in the condensation of the anticoagulant-fibrinogen dissolution system. Alpha enolase can combine with plasminogen, affecting the fibrinolytic system of the body. Apolipoprotein A-I can inhibit blood clotting by promoting fibrinolysis and reducing platelet aggregation. In cows, adhesion between the foetal and maternal placental tissues can occur after the rupture of the umbilical cord. This results in restricted blood clotting, which prevents the separation of the placental tissues and subsequent delivery of the placenta.

When the birth canal is damaged during delivery, inflammatory reactions can occur within the body of the cow. Inflammation of the placenta causes lesions in the foetal placental tissues, connective tissue hyperplasia, and placental tissue congestion. Under these conditions, the maternal placenta adheres to the foetal placenta, which is disadvantageous to the separation and delivery of the placenta. Therefore, inflammation is a key factor in the incidence of bovine RFM. Both apolipoprotein A-I and alpha enolase, which were identified in our experiment, control the inflammatory reaction. Alpha enolase is a metabolic enzyme that participates in glycolysis and can interact with plasminogen to affect the fibrinolytic system, enabling pathogenic microorganisms to infect the body. The increased expression of alpha enolase promotes the infection of placental tissues by pathogenic microorganisms that cause inflammation of the interface maternal placental tissues, interfering with the separation of the afterbirth. Because apolipoprotein A-I negatively controls the inflammatory reaction and the secretion of inflammatory factor IL-1β (Stein, 1999), the increased expression of apolipoprotein A-I may be caused by inflammatory reactions that occurred during birth, thus giving rise to RFM in the cows. The inhibition of the inflammatory reaction may result in the increased expression of apolipoprotein A-I. Aldose reductase is a key rate-limiting enzyme of the polyhydric alcohol pathway which has recently been verified to participate in the inflammatory reaction. Other studies indicated that serpin protease inhibitor could affect the progress of inflammation (Huntington, 2011). Therefore, this study predicted that serpin protease inhibitor might participate in the progress of RFM.

When the ability to scavenge free radicals is weakened, the damaged maternal placenta adheres to the foetal placenta due to the assault by excessive oxygen radicals to the cytomembrane. Thus, RFM occurs because the removal of the microvilli from the recesses of caruncula on the foetal placenta is prevented. Heat shock proteins are protective proteins that are expressed abundantly in the body under conditions of oxidative stress or heat stress. Under these conditions, HSP27 has the physiological function of combining with and activating the PKB/AKT/RAC pathway, inhibiting the function of NADPH oxidase in the body, reducing the level of ROS in the cells, and reducing any rouging effects (Garrido et al., 2006). The expression of HSP27 is decreased in the RFM maternal placentas, resulting in miopragia of the body's antioxidant defences and the occurrence of RFM. Glutathione transferase is widespread in the body and can act as an antioxidant that participates in the process of detoxification. Glutathione transferase physiologically functions as an antioxidant (Vranová et al., 2002).

Transketolase is a rate-limiting enzyme in the pentose phosphate pathway, and NADPH, which is produced by transketolase through the pentose phosphate pathway, functions as an antioxidant. In our experiment, the expressions of glutathione transferase and transketolase were increased, suggesting that glutathione transferase and transketolase may participate in the genesis and development of RFM through the antioxidant functions of their downstream products.

Recently, many scholarly studies have shown the relationship between minerals and vitamins and the delivery of the afterbirth, indicating that nutrition metabolism is related to RFM. Apolipoprotein A-I and aldose reductase have been shown to affect fat metabolism by participating in the metabolism of glycerides and the digestive absorption of fat. Research has also shown that the metabolism of dairy cows during the perinatal period can affect the production performance of the cows, which can affect the incidence of RFM in cattle. However, the mechanism for how this occurs has not yet been reported. In our experiments, it was found that the expression of alpha-enolase was decreased in the maternal placental tissues of the RFM group. This may result in energy deficiencies, weaker uterine contractions, and subsequent difficulty in delivering the afterbirth. Apolipoprotein A-I participates in the digestion and absorption of vitamins. Previous research has shown that in cattle the plasma levels of vitamins D and E decrease during RFM; a result that has been corroborated by our study.

Conclusion

This study obtained differential expressions of proteins between RFM and normal foetal membrane using comparative proteomics. These proteins participate in the processes of blood clotting, energy metabolism, inflammation, and oxidative stress, respectively. All of these processes play

important roles in RFM. Therefore, the differential expressions of proteins obtained in this study may participate in the process of RFM.

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บทคัดย่อ

การวิเคราะห์เปรียบเทียบทางโปรตีนโอมิกในโคนมที่มีภาวะรกค้าง

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ภาวะรกค้าง หรือ Retained foetal membrane (RFM) เป็นพยาธิสภาพทางระบบสืบพันธุ์ที่มีความสำคัญในโคนม การศึกษาครั้งนี้มีวัตถุประสงค์ เพื่อศึกษาพยาธิวิทยาของภาวะรกค้าง และคัดกรองหาตัววัดวิเคราะห์ทางคิลนิก โดยวิธีการเปรียบเทียบทางโปรตีนโอมิก วิธีการศึกษาโดยเก็บตัวอย่างรก จากโคนมที่มีภาวะรกค้างจำนวน 3 ตัวอย่าง และกลุ่มควบคุม 3 ตัวอย่าง จากการวิเคราะห์ทางโปรตีนโอมิกเบื้องต้นพบว่า ในตัวอย่างรกของลูก (foetal placental tissue) มีโปรตีนจำนวน 240 จุดที่มีความแตกต่างระหว่างกลุ่มศึกษา และในตัวอย่างรกของแม่ (maternal placental tissue) มีโปรตีนจำนวน 214 จุดที่มีความแตกต่างระหว่างกลุ่มศึกษา โดยที่ในตัวอย่างรกของลูก สามารถแยกโปรตีนได้จำนวน 10 ชนิด โดยพบว่าโปรตีน 7 ชนิดมี expression ของโปรตีนอย่างมีนัยสำคัญ ได้แก่ BSA, alpha enolase, apolipoprotein A-I, annexin A8-like 1, serine proteinase inhibitor, glutathione transferase, and transketolase ส่วนในตัวอย่างรกของแม่ สามารถแยกโปรตีนได้จำนวน 9 ชนิด โดยพบว่าโปรตีน 5 ชนิดมี expression ของโปรตีนอย่างมีนัยสำคัญ ได้แก่ aldose reductase, heat shock 27 kDa protein 1, serine (or cysteine) proteinase inhibitor, alpha enolase, and annexin A2) สาเหตุและภาวะโน้มนำของการเกิดโรคนี้ อาจเกี่ยวข้องกับภาวะ fibrinolysis, pyruvate metabolism, inflammatory response และ oxidative stress

คำสำคัญ: โคนม รกค้าง โปรตีนโอมิก ตัววัดวิเคราะห์ทางคลินิก

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