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Differential Expression of Bovine Leucocyte Antigen Class I Chain-Related Genes 2 in preganant Dairy Cows and Neonatal Calves

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Abstract: Major histocompatibility complex takes the capital contribution to maternal immune responses to the semiallogenic fetus. As the member of BoLA-Ib, the machenism of class I chain-related genes 2 (*MIC2*) on pregnant setup and delivery, till remains to be elucidated. To evaluate the possible effect of MIC2 on pregnant establishment and delivery, neonatal ear tissues, fetal placenta, peripheral blood Polymorphonuclear Neutrophils (PMN) of dairy cow in different pregnant stages were collected to analyze MIC2 expression by quantitative RT-PCR. MIC2 on PMN in dairy cows was down regulated expressed in 1st and 2nd trimesters and recoveried back to normal level in peripartum. Moreover, MIC2 was novel upregulated expressed in neonatal ear tissues and downregulated expressed in fetal placenta. These results suggested that MIC2 lowly expressed in the 1st and 2nd trimester of pregnant cows could suppress NKG2D binding MIC2 on PMN and therefore, let embryo escape from maternal immune response. Anabiotic MIC2 expression in peripartum suggested that maternal immune to full grown foetus recover back to normal level which be in favour of delivery. In foetus, novel upregulated expression of MIC2 in tissues was favourible for foetus to escape its autoimmunity. Whereas, downregulated expression in fetal placenta let placenta decoherenced from maternal placentome which was beneficial to delivery.

Key words: MIC2, pregnant dairy cow, neonatal calf, polymorphonuclear neutrophils, fetal placenta, China

INTRODUCTION

The maternal immune system is in close contact with the cells and tissues of fetus. MHC is a large gene family in most vertibrates and takes the capital contribution to maternal immune responses to semiallogenic fetus during pregnancy (Apps *et al*., 2008; Schafer-Somi *et al*., 2009). MHC-I in bovine are named as Bovine Leucocyte Antigen 1 (BoLA-I) and grouped as classic molecules (BoLA-Ia) and non classic molecules (BoLA-Ib) by the BoLA nomenclature committee of the International Society for Animal Genetics. Actually, consistent expression of all BoLA-I in cattle may be unusual because interlocus recombination occurs in cattle BoLA-I and even the precise number of BoLA-I loci are not known (Ellis, 2004; Holmes *et al*., 2002). Major histocompatibility complex class I chain-related Molecules (MIC) is belonged to non-classic MHC-I (Holmes *et al*., 2002; Trowsdale, 1995; Zwirner *et al*., 1998) and has three genotypes, MICA, MICB and a pseudogene in most species (Bahram, 2000; Chardon *et al*., 2000). Three essentially complete MIC in cattle (Bos taurus) located on a single contig (NW_001494163) within the BoLA-I region of chromosome 23 have been provisionally named as BoLA MIC1, BoLA MIC2 and BoLA MIC3 (Birch *et al*., 2008). The intron sizes between the three BoLA MIC are remarkably similar to one another and even show a similar pattern to human MIC (Bahram *et al*., 1994). MIC is bound tightly with Natural Killer cell Group 2D (NKG2D) to eliminate pathogen-infected (or tumour) cells while prevent the killing normal cells (Mistry and O'Callaghan, 2007). NKG2D had been identified in cattle (Fikry *et al*., 2007) and NKG2D ligands genes had also discovered as MIC (Larson *et al*., 2006).

MIC expression had been reported on epithelial, endothelial cells, transplanted cell and tumours (Collins, 2004). Although, some reports presumed that MIC was not present on $CD4^+$ and $CD8^+$ T cells and B cells (Collins *et al*., 2005), MICB expression on PMN was applied to evaluate the prognosis of B-cell chronic lymphocytic leukemia in human (Nuckel *et al*., 2010). MIC are linkage disequilibrium with HLA-B (Collins, 2004) and has important effect on semiallogenic immune or pretection dring gestation in human (Trowsdale, 1995).

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Diary cow semiallogenic immune or pretection occurs dring gestation but there are currently no functional data relating to cattle MIC (Birch *et al*., 2008). Cause of their broad similarity to human MIC in terms of genomic location, gene structure and limited expression profile, cattle MIC may play an equivalent role with human MHC (Trowsdale, 1995; Birch *et al*., 2008; Marsili *et al*., 2010). Moreover, the structual and functional properties of MICB are similar to those of MICA (Steinle *et al*., 2001). The aims of this study were to detect MIC2 relative expression quantity on PMN in pregnent dairy cows and further reveal its expression regularity in trimester of pregnancy. Meanwhile, the possible function of MIC2 on fetal protectiom or delivery was deduced with its expression characteristics in fetal placenta and neonatal tissues.

MATERIALS AND METHODS

Animals: About 10 pregnant Holstein diary cows (2-3 parity) were reared with basic feeds and forage and milked three times every day in the experiment farm of Guangming Milk Cooperation (Shanghai, China). The average milk yield was 35 kg in 1st trimester and 30 kg in 2nd trimester and the last trimester were in delactation. About 10 healthy replacement heifer without gestation were reared routinely and used as control. Blood were sampled, respectively in different trimesters from same cow and in the last time, fetal placenta and ear tissues of neotatal calf were collected from the above cows in 1 h postparturition (Table 1). All cows were diagnosed and did not show any clinical symptom, especially any symptom of ovary, uteri and mammary diseases.

Preparation of PMN, ear tissues and fetal placenta: Anticoagulated blood were sterilely collected to isolate PMN in 1 h. About 4 mL pH 7.2 erythrocyte lysate (NH_4Cl 8.30 g L^{-1}, Tris 2.0594 g L^{-1}, adding ddH_2O 1,000 mL and autoclaving) (Mebus and Underdahl, 1997) was added into 1 mL anticoagulated blood and homogenized for 10-15 min and then centrifuged at 3,000 r min^{-1} for 10 min. Above manipulation was repeated tricely till the sediemnts appeared without erythrocyte. Sediments was mixed with 2 mL pH 7.4 phosphate buffered solution (NaCl 8.00 g L^{-1}, KCl 0.20 g L^{-1}, Na_2HPO_4 1.44 g L^{-1} and KH_2PO_4 0.24 g L^{-1} adding double distilled water to 1,000 mL and autoclaving (Mebus and Underdahl, 1997) which was prepared with ddH_2O and dealed with diethyl pyrocarbonate and centrifuged again at 8000 rpm min^{-1} for 40 sec. The last sediments was PMN. PMN were suspensed with pH 7.4 phosphate buffered solution and adjusted to about 8.0×10^9 L^{-1}.

Table 1: Fetal placenta, neanatal ear tissues and blood in different pregnant stages of dairy cows

Samples obtained from	Samples amount	Sample tissues	Description
Replacement heifers*	10	Blood	2 years old, no pregnancy
First trimester of pregnancy#	10	Blood	Gestational age 55±10 days
Second trimester of pregnancy#	10	Blood	Gestational age 143±10 days
Peripartum#	10	Blood	6±4 days antepartum
Fetal placenta#	10	Fetal placenta	1 h postpartum
Neonatal calves#	10	Ear tissues	1 h postpartum
Total	60		

*Replacement heifers were applied as control because the RT-PCR was incomplete quantitative method. In statistics, the MIC2 RNA on PMN in replacement heifers was regarded as 1.000 and all the others were normalized according to it. #The other samples were took from the same pregnant cows throughout gestation (Cohort longitudinal design)

Ear tissues were cliped down from the inferior border of neonatal calve's ear in 30 min postpartitum. Blood in ear tissues and placenta was squeezed out and rinsed with pH 7.4 PBS and then comminuted with scissors.

PMN, fetal placenta and ear tissues were mixed with TRIzol® (Invitrogen, Carlsbad, CA, USA) and snap frozen in liquid nitrogen immediately and then took back to lab and stored at -80°C for the use of RNA isolation.

RNA isolation and reverse transcription: PMN, placenta and ear tissues were schizolysed with cell lysate and RNA was extracted according to the operating instruction of RNAPure reagent kit (BioTeke Corporation, China, RP1202). About 16 μL RNA was diluted to 600 μL and quantitatied by ultraviolet spectroscopy (Beckman, DU640), the photoabsorption at A260/A230 was 2.0 and all the concentration was set up to 4 μg/6 μL. RNA (5 μL) was forward confirmed on 1% agarose gel with 300 voltage for 8 min. Cause of that the cell numbers of fetal placenta and neonatal tissues could not be count, RNA from all tisssues must be adjusted to a equivalent level for RNA quantitation.

RNA from all samples were treated with RNAse-free DNAse and SuperScript™ III reverse transcriptase (BioTeke super RT kit, PR6601) with Oligo (DT) primers for reverse transcription. About 6 μL of RNA was mixed with 1 μL Olig (DT) primers, 2 μL dNTP mixture (dNTP mixture, Toyobo, 82650G6) and 4 μL RNAse free ddH_2O and reacted in PCR, Applied Biosystems, Model 9902, USA at 65°C for 5 min and then quenched for 2 min on ice. The above solution was mixed forward with 1 μL reverse transcriptase M-MULV (200 U μL^{-1}, Fermentas, 00033507), 4 μL 5× first-strand buffer and 1 μL RNAse inhibitor and reacted in PCR at 30°C for 10 min, 42°C for 60 min and 95°C for 5 min. The last product was cDNA. The forward and reverse primers of cattle house-keeping gene (*Bos taurus* β-actin, ACTB), respectively were

F 5'-TGGACTTCGAGCAGGAGAT-3' and R 5'-CGTCACAC TTCATGATGGAA-3' (objective segment size 194 bp) and were used to evaluate the effect of RNA isolation and reverse transcription. The reaction system of Bos taurus β-actin was Taq (5 μL) 0.3 μL, Buffer 2 μL, dNTP (10 mM) 0.3 μL, cDNA 1 μL, primer F (10 μM) 1 μL, primer R (10 μM) 1 μL and ddH_2O 14.4 μL. The above solution was reacted in PCR at 95°C for 5 min, 95°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec for total 35 circulations and forward on 70°C for 10 min. cDNA was confirmed by electropheresis on 1% agarose gel with 300 V for 8 min.

BoLA MIC2 sequencing and sequence analysis: Primers were designed with software DNAMAN according to the BoLA MIC2 sequence in GenBank. The forward and reverse primer of BoLA MIC2 was respectively M1 5'-AGAAAGGAGGCTTACATTCCC-3'and M2 5'-GCCTGG TAATGCTTGCTTAAC-3' (objective segment size 199 bp). Taq (5 u μL^{-1}) 0.5 μL, Buffer 5 μL, dNTP, 10 mM) 0.75 μL, M1 (10 uM L^{-1}) 2 μL, M2 (10 uM L^{-1}) 2 μL, ddH_2O 39.75 μL and cDNA 6 μL were mixed and reacted with PCR (Applied Biosystems, Model 9902, USA) and the reacted condition was same as the primers of ACTB. The above solution was amplificated in PCR at 95°C for 5 min, 95°C for 30 sec, 54°C for 30 sec and 72°C for 30 sec for total 35 circulations and forward on 70°C for 10 min.

Amplificated segment was seperated by electropheresis on 1% agarose gel with 300 voltage for 8 min, objective segment was cut down and recovered with Gel purification kit (Spin-column) (BioTeke, DP1601). The recovered gene segment was connected with pMD-18-T vector (TAKARA) and transfected into competent cell DH5α. DH5α was cultured at 37°C for 12 h. MIC2 segment was amplificate from DH5α with the primer M1 and M2 on above condition. About 5 μL amplificated solution was electropherized on 1% agarose gel with 300 voltage for 8 min. All amplificated products were sequenced and compared with MIC2 in NCBI blast while product size in agarose gel was similar with objective gene size.

Real-time PCR analysis of MIC2 expression: cDNA was diluted 10 times. MIC2 primer (same as above) M1 0.2 μL, MIC2 primer M2 0.2 μL, candidate reference genes *ACTB* primer F1 0.2 μL, candidate reference genes *ACTB* primer F2 0.2 μL, dNTP mixture 12.5 μL, purified cDNA 2 μL and sterilizing distilled water were mixed to analyzed MIC2 expression by RT-PCR (bioer-FDQ48A, USA) with the reagents of SYBR green (Invitrogen). The reactive conditions were at 95°C for 5 min, 95°C for 30 sec, 54°C for 30 sec and 72°C for 30 sec for total 35 circulations and forward on 70°C for 10 min.

Statistics: The data of real-time RT-PCR were analyzed by the Cycle threshold (Ct) method (Livak and Schmittgen, 2001). MIC2 expression quantitation ($2^{-\Delta\Delta CT}$) on PMN of replacement heifers was set as 1.000, data from other samples were normalized separately according to it. Afterward, corrected Ct data of MIC2 expression quantitation in differrent groups were compared using Student t-test.

RESULTS AND DISCUSSION

RNA were successfully isolated (Fig. 1), reversely transcribed to cDNA (Fig. 2) and MIC2 was successfully amplified from transfected pMD-18-T-DH5α (Fig. 3). The amplificated segment from transfected DH5α was sequenced and compared and the concordance rate was 100% with the objective gene *MIC2* in NCBI blast (Accession No. GI: 187937198) (Fig. 4).

Table 2 showed the differential expression of MIC2 on PMN in different trimesters of diary cows. Compared with in replacement heifers, MIC2 were lower on PMN in 1st and 2nd trimesters ($p<0.0001$) but no different with in peripartum ($p>0.05$).

A general tendency of MIC2 expression on PMN was fall-off in 1st and 2nd trimesters of pregnancy and recoveried back in peripartum. The novel upregulated expression of MIC2 in neonatal ear tissues was >300 times in any other cows ($p<0.0001$).

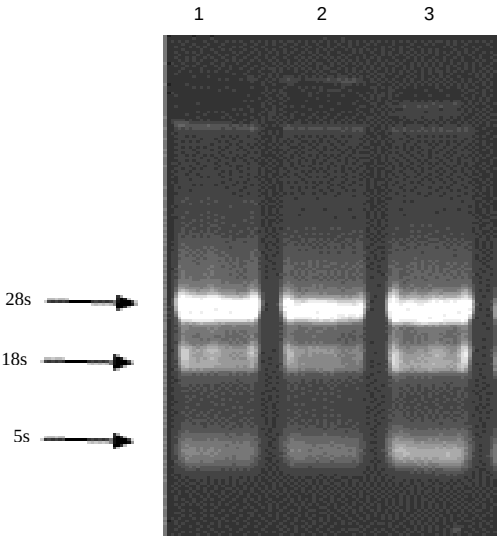

Fig. 1: RNA isolated from samples on 1% agarose gel electrophoresis; 1: PMN from replacement heifer; 2: PMN from pregnant cow in 1st trimester and 3: neonatal calves ear tissue. The bright segments from up to down were 28sRNA, 18sRNA and 5sRNA

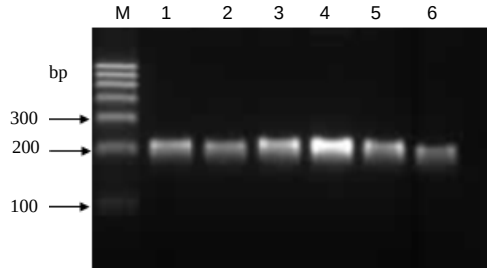

Fig. 2: cDNA reversely transcribed and amplified with primers of *Bos taurus* β-actin on 1% agarose gel electrophoresis. The segments from right to left were Marker (M), cDNA from replacement heifer (1); cDNA from pregnant cows in first (2), second (3) and the last (3) trimesters; cDNA from neonatal ear tissue (4) and fetal placenta (5). All of the cDNA segments were at the objective segment size 194 bp

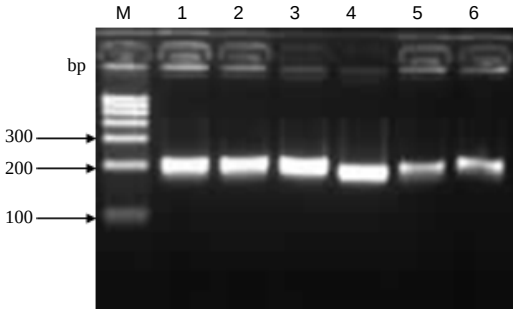

Fig. 3: MIC2 amplificated from pMD-18-T-DH5α. The segments from right to left were Marker (M); MIC2 from replacement heifer (1); MIC2 from pregnant cows in first (2), second (3) and the last (3) trimesters; MIC2 from neonatal ear tissue (4) and fetal placenta (5). All of the MIC2 segments were at the objective segment size 199 bp

Inversely, it was down regulated even silenced in fetal placenta (Table 3). MIC expression had been reported on epithelial, endothelial cells, transplanted cell and tumours (Collins, 2004). Although, some reports presumed that MIC was not present on $CD4^+$ and $CD8^+$ T cells and B cells (Collins *et al*., 2005; Zwirner *et al*., 1999), MIC2 expression on peripheral blood lymphocytes was also applied to evaluate the prognosis of B-cell chronic lymphocytic leukemia in human (Nuckel *et al*., 2010). The fact that PMN were isolated from blood and used for MIC2 quantitative expression by RT-PCR in this test suggested that MIC2 express on PMN.

Table 2: Quantitative expression of MIC2 on PMN in different trimesters of pregnant cows

Samples obtained from	n	$\Delta\bar{C}T$	$\Delta\Delta^{\bar{C}T}$	$2^{-\Delta\Delta\bar{C}T}$	$SE^{(2-\Delta\Delta CT)}$
Replacement heifers (control)	10	9.832	0.000	1.000^a	0.271
First trimester of pregnancy (trial)	10	11.918	2.086	0.249^b	0.029
Second trimester of pregnancy (trial)	10	12.327	2.495	0.182^b	0.011
Pregnant cows in peripartum (trial)	10	10.160	0.328	0.804^a	0.009

*The same letter on the right shoulder of data in the $2^{-\Delta\Delta CT}$ colunm meant non-significant difference ($p>0.05$) and different lower case letters meant significant difference ($p<0.05$). *CT: The circulation of objective gene amplificated to setting threshold; * $\Delta\bar{C}T$ = CT (objective gene) - CT (house-keeping gene); * $\Delta\Delta^{\bar{C}T} = \Delta\bar{C}T$ (trial) - $\Delta\bar{C}T$ (control); * $2^{-\Delta\Delta\bar{C}T}$: The multiples of objective gene in trial than that in control; *$SE^{(2-\Delta\Delta CT)}$: Standard Error

Table 3: Quantitative expression of MIC2 in neonatal ear tissue and fetal placenta of dairy cows

Samples obtained from	n	$\Delta\bar{C}T$	$\Delta\Delta^{\bar{C}T}$	$2^{-\Delta\Delta\bar{C}T}$	$SE^{(2-\Delta\Delta CT)}$
Replacement cows (control)	10	9.832	0.000	1.000^A	0.271
Fetal placenta (trial)	11	12.726	2.894	0.140^B	0.012
Neanatal calves (trial)	7	1.601	-8.23	309.707^C	31.639

*The different capital letter on the right shoulder of data in the $2^{-\Delta\Delta CT}$ colunm meant extremely significant difference ($p<0.01$). *CT: The circulation of objective gene amplificated to setting threshold. *$\Delta\bar{C}T$ = CT (objective gene) - CT (house-keeping gene); * $\Delta\bar{C}T = \Delta\bar{C}T$ (trial) - $\Delta\bar{C}T$ (control); * $2^{-\Delta\Delta\bar{C}T}$: The multiples of objective gene in trial than that in control; *$SE^{(2-\Delta\Delta CT)}$: Standard Error

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 Score =  368 bits (199),  Expect = 2e-106
 Identities = 199/199 (100%), Gaps = 0/199 (0%)
 Strand=Plus/Plus

Query  1    AGAAAGGAGGCTTACATTCCCTCCAGGAGACCGTGGGCTGTGATATCAATGAAGACAGCC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  379  AGAAAGGAGGCTTACATTCCCTCCAGGAGACCGTGGGCTGTGATATCAATGAAGACAGCC  438

Query  61   ACCCCCAGGGCTTCCGGCTTCTCTACTTCAATGGGGAGCTCCTCCTCTCCTGCTACCCGG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  439  ACCCCCAGGGCTTCCGGCTTCTCTACTTCAATGGGGAGCTCCTCCTCTCCTGCTACCCGG  498

Query  121  AGCCCCACGGATGTACCCTGCCCCAGTCCTCGGCTCGGACCTTGGCCATGGAAATGGAGT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  499  AGCCCCACGGATGTACCCTGCCCCAGTCCTCGGCTCGGACCTTGGCCATGGAAATGGAGT  558

Query  181  TAAGCAAGCATTACCAGGC  199
            ||||||||||||||||||
Sbjct  559  TAAGCAAGCATTACCAGGC  577
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Fig. 4: Comparision of clone segment with MIC2 in NCBI blast. The objective gene was 199 bp, concordance rate was 100%, gaps was zero

In human, no protein expression of MICA and MICB or HLA-F could be detected in decidual leukocytes from the 1st trimester of pregnancy and thus suggested that the role of NKG2D is not focussed on trophoblast recognition in normal pregnancy (Apps *et al*., 2008). Although, bovine MHC expression is somewhat different with in human (Davies *et al*., 2006), the low expression of MIC2 on PMN in the 1st and 2nd trimester of pregnancy in dairy cows, still suggested that PMN cytotoxicity was suppressed by NKG2D binding less MIC2 and then let embryo escape from maternal immune response. The intriguing counterevideace of this immuno-suppression phenomenon was that MIC2 expression on PMN in peripartum cows was recoveried back to the level of replacement heifers in this test which was in favour of delivery.

MIC2 are induciblly expressed on stressed cell surface (Borchers *et al*., 2009). Notably, human MICA and MICB was high expressed on the apical and basal cell membrane of uterus and on exosomes (embryo and placenta) and these expression in the syncytiotrophoblast was profit to placenta formation and let embryo escape from maternal immune response in the first trimester (Mincheva-Nilsson *et al*., 2006). Tumor, the abnormal tissue can induce itself expressing MICB and therefore interfering and suppressing the activity of NK cell (Konjevic *et al*., 2010).

Actually, MHC antigen expressed on the bovine placenta and their differential expression were relative to abnormal pregnancies and retained placenta (Davies *et al*., 2004). Engagement of these data and the fact that MIC2 was strangely up-regulated expressed in neonatal ear tissue and down regulated in fetal placenta, researchers deduced that upregulated expression of MIC2 in tissues was helpful for tissue escaping from fetal autoimmunity. Although, nonage fetal placenta did not be analyzed, the down regulated expression in late fetal placenta which was discharged by maternal immune response should partly verify that the exuviated fetal placenta was simultaneously rejected by both maternal and fetal immunological system.

CONCLUSION

The present study showed that MIC2 lowly expressed in the 1st and 2nd trimester of pregnant cows could suppress NKG2D binding MIC2 on PMN and therefore, let embryo escape from maternal immune response. Anabiotic MIC2 expression in peripartum suggested that maternal immune to full grown foetus recover back to normal level which be in favour of delivery.

In foetus, novel upregulated expression of MIC2 in tissues was favourible for foetus to escape its autoimmunity. Whereas, downregulated expression in fetal placenta let placenta decoherenced from maternal placentome which was beneficial to delivery.

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REFERENCES

Apps, R., L. Gardner, J. Traherne, V. Male and A. Moffett, 2008. Natural-killer cell ligands at the maternal-fetal interface-UL-16 binding proteins, MHC class-I chain related molecules, HLA-F and CD48. Human Reprod., 23: 2535-2548.

Bahram, S., 2000. MIC genes: From genetics to biology. Adv. Immunol., 76: 1-60.

Bahram, S., M. Bresnahan, D.E. Geraghty and T. Spies, 1994. A second lineage of mammalian MHC class I genes. Proc. Natl. Acad. Sci., 91: 6259-6263.

Birch, J., C. De Juan Sanjuan, E. Guzman and S.A. Ellis, 2008. Genomic location and characterisation of MIC genes in cattle. Immunogenetics, 60: 477-483.

Borchers, M.T., S.C. Wesselkamper, V. Curull, A. Ramirez-Sarmiento and A. Sanchez-Font *et al*., 2009. Sustained CTL activation by murine pulmonary epithelial cells promotes the development of COPD-like disease. J. Clin. Investigation, 119: 636-649.

Chardon, P., C. Rogel-Gaillard, L. Cattolico, S. Duprat, M. Vaiman and C. Renard, 2000. Sequence of the swine major histocompatibility complex region containing all non-classical class I genes. Tissue Antigens, 57: 55-65.

Collins, R.W.M., 2004. Human MHC class I chain related (MIC) genes: Their biological function and relevance to disease and transplantation. Eur. J. Immunogenet, 31: 105-114.

Collins, R.W., H.A. Stephens, M.A. Clare and R.W. Vaughan, 2005. High resolution molecular phototyping of MICA and MICB alleles using sequence specific primers. Human Immunol., 63: 783-794.

Davies, C.J., J.R. Hill, J.L. Edwards, F.N. Schrick, P.J. Fisher, J.A. Eldridge and D.H. Schlafer, 2004. Major histocompatibility antigen expression on the bovine placenta: Its relationship to abnormal pregnancies and retained placenta. Anim. Reprod. Sci., 82-83: 267-280.

Davies, C.J., J.A. Eldridge, P.J. Fisher and D.H. Schlafe, 2006. Evidence for expression of both classical and non-classical major histocompatibility complex class i genes in bovine trophoblast cells. Am. J. Reproductive Immunol., 55: 188-200.

Ellis, S., 2004. The cattle major histocompatibility complex: Is it unique?. Vet. Immunol. Immunopathol., 102: 1-8.

Fikry, Y., J. Nyabenda, J. Content and K. Huygen, 2007. Cloning, sequencing and cell surface expression pattern of bovine immunoreceptor NKG2D and adaptor molecules DAP10 and DAP12. Immunogenetics, 59: 653-659.

Holmes, M.A., P. Li, E.W. Petersdorf and R.K. Strong, 2002. Structural studies of allelic diversity of the MHC class I homolog MIC-B, a stress-inducible ligand for the activating immuno receptor NKG2D. J. Immunol., 169: 1395-1400.

Konjevic, G., K.M. Martinovic, A. Vuletic and N. Babovic, 2010. *In vitro* IL-2 or IFN-á-induced NKG2D and CD161 NK cell receptor expression indicates novel aspects of NK cell activation in metastatic melanoma patients. Melanoma Res., 20: 459-467.

Larson, J.H., B.M. Marron, J.E. Beever, B.A. Roe and H.A. Lewin, 2006. Genomic organization and evolution of the ULBP genes in cattle. BMC Genomics, 7: 227-241.

Livak, K.J. and T.D. Schmittgen, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\hat{A}\hat{A}C}$ T method. Methods, 25: 402-408.

Marsili, L., S. Maltese, L. Carletti, D. Coppola, S. Casini and M.C. Fossi, 2010. MICA expression as toxicological stress marker in fibroblast cell cultures of cetaceans. Comp. Biochem. Physiol. Part A, 157: S22-S28.

Mebus, C.A. and N.R. Underdahl, 1997. Seanning eleetron microscopy of traehea and bronchi from gnotobiotic pigs inoeulated with *Mycoplasma hyopneumonia*. Am. J. Vet. Res., 38: 1249-1254.

Mincheva-Nilsson, L., O. Nagaeva, T. Chen, U. Stendahl and J. Antsiferova *et al*., 2006. Placenta-derived soluble MHC class I chain-related molecules down-regulate NKG2D receptor on oeripheral blood mononuclear cells during human pregnancy: A possible novel immune escape mechanism for fetal survival. J. Immunol., 176: 3585-3592.

Mistry, A.R. and C.A. O'Callaghan, 2007. Regulation of ligands for the activating receptor NKG2D. Immunology, 121: 439-447.

Nuckel, H., M. Switala, L. Sellmann, P.A. Horn and J. Durig *et al*., 2010. The prognostic significance of soluble NKG2D ligands in B-cell chronic lymphocytic leukemia. Leukemia, 24: 1152-1159.

Schafer-Somi, S., H.B. Beceriklisoy, I. Walter, S. Sabitzer and D. Klein *et al*., 2009. Expression of MHC-I and -II in uterine tissue from early pregnant bitches. Reprod. Domestic Anim., 44: 103-108.

Steinle, A., P.W. Li, D.L. Morris, V. Groh, L.L. Lanier, R.K. Strong and T. Spies, 2001. Interactions of human NKG2D with its ligands MICA, MICB, and homologs of the mouse RAE-1 protein family. Immunogenetics, 53: 279-287.

Trowsdale, J., 1995. Both man and bird and beast: Comparative organization of MHC genes. Immunogenetics, 41: 1-17.

Zwirner, N.W., M.A. Fernandez-Vina and P. Stastny, 1998. MICA, a new polymorphic HLA-related antigen, is expressed mainly by keratinocytes, endothelialcells and monocytes. Immunogenetics, 47: 139-148.

Zwirner, N.W., K. Dole and P. Stastny, 1999. Differential surface expression of MICA by endothelial cells, fibroblasts, keratinocytes and monocytes. Hum. Immunol., 160: 323-330.