Journal of Reproduction and Development, Vol. 58, No 5, 2012

—Original Article—

Non-classical Major Histocompatibility Complex Class Makes a Crucial Contribution to Reproduction in the Dairy Cow

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Abstract. The aim of this study was to evaluate the effect of classical and non-classical major histocompatibility complex (MHC) on the reproduction in the dairy cow. Nine pairs of *MHC-I* genes were chosen according to their homology and possible function, and their transcription levels in maternal peripheral blood mononuclear cells (PBMCs) from all three trimesters and transcription levels in fetal tissues were compared to evaluate their contributions to cattle reproduction. The results showed that three non-classical genes were variably expressed in PBMCs of pregnant cows. *MICB* was downregulated in the first and second trimesters ($P<0.05$), but recovered back to the level in replacement heifers in the last trimester ($P>0.05$). *BoLA-NC1** was upregulated in the first and last trimesters ($P<0.001$) but no different in the second trimester ($P>0.05$). *BoLA-NC3** was upregulated in all trimesters ($P<0.001$). On the other hand, *MICB* was upregulated in fetal ear tissues ($P<0.001$), and *BoLA-NC1** was almost silent in both fetal placenta and ear tissues ($P<0.001$); however, *BoLA-NC3** was upregulated in both the fetal placenta and ear tissues ($P<0.001$). These results suggested that non-classical gene *BoLA-NC1** increased maternal immunity against the fetus, which was inhibited by *BoLA-NC3**. *BoLA-NC3** also inhibited fetal autoimmunity. Apoptosis of the fetal placenta could reduce itself expressing *MICB*, and upregulated expression of *MICB* in ear tissues was favorable for the fetus to escape autoimmunity. On the other hand, downregulated expression of *MICB* in the fetal placenta allows for placental decoherence from the maternal placentome, which was beneficial to fetus delivery. Although classical genes were expressed differentially, their effects were restricted because of heavy chain deficiency.
Key words: Diary cow, *MHC-I*, Reproduction

(**J. Reprod. Dev. 58:** 569–575, 2012)

Major histocompatibility complex (MHC) is a large gene family in most vertebrates, and makes crucial contribution to immune responses [1–6]. Similar to human MHC, MHC-I in the bovine is further categorized as classical molecules and non-classical molecules [7]. Classical *class I* genes are highly polymorphic and expressed in most somatic cells, and these class proteins present peptides from the animal own proteins or from intracellular pathogens to T cell receptors on $CD8^+$ and cytotoxic suppressor T lymphocytes. Furthermore, classical class I proteins serve as the ligand for inhibitory receptors on natural killer (NK) cells and other leukocytes [7–9]. Non-classical class I genes are monomorphic or oligomorphic and have restricted cellular expression [8]. Although non-classical class I proteins have diverse functions, one important function is to act as ligands for inhibitory leukocyte receptors, including receptors encoded in the natural killer cell group 2D (NKG2D) complex and the leukocyte receptor complex [10–12].

A fetus containing parental *MHC-I* genes is a semi-allograft to the dam. So the immunotolerance of the dam to the fetus is the essential demand for pregnancy establishment and maintenance [5]. This immunotolerance is set up by the differential expression of MHC class I in trophoblastic cells in many species animals [13, 14]. The MHC-I expressed in cattle is different from that in human [8]. Mature trophoblast cells of most species do not express classical MHC class I proteins [15, 16]. However, it is normal for bovine trophoblast cells in the interplacentomal and arcade regions of the placenta to express classical and non-classical MHC-I proteins during the last trimester of pregnancy [13, 17]. These differences do not have an adverse effect on pregnancy and conversely are probably beneficial to pregnancy establishment in cattle [18]. MHC-I could also be expressed in PBMCs [13, 19–21]. Recently, a number of reports have mainly focused on the expression of MHC-I in both trophoblasts and dam blood, and further, systemic samples from different pregnancy stages and fetuses of dairy cows have not been applied to investigate MHC-I differential transcription and therefore elucidated their effect on pregnancy establishment and fetus delivery.

MHC-I is highly polymorphic; thousands of *Bos taurus MHC-I* gene hits and antigens have been reported in GenBank and NCBI, and their total is still increasing. Actually, analyzing the transcription of all these genes in cattle may be unusual, because the precise numbers of *MHC-I* loci are still not known [22], and especially because *MHC-I* genes are subjected to interlocus recombination [23] and new mutant sites may emerge in different species and individuals [24]. To elucidate their effect on pregnancy establishment and fetus delivery

Received: November 8, 2011
Accepted: May 22, 2012
Published online in J-STAGE: June 29, 2012

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in dairy cows, nine pairs of *MHC-I* genes (three non-classical genes, six classical genes containing a heavy chain gene) were chosen to compare their transcription levels in the fetal ear tissues and placenta, and in maternal PBMCs at different pregnant stages.

Materials and Methods

Animals

Ten pregnant Holstein diary cows (2 parities) were reared with basic feeds and forage and milked three times every day at the experiment farm of Guangming Milk (Shanghai, China). The average milk yield was 35 kg in the first trimester and 30 kg in the second trimester, and the cows were delactation in the last trimester. Ten healthy replacement heifers were reared routinely and used as controls. Anticoagulated blood was sampled sterilely from the caudal vein in different trimesters from the same cows, and final blood samples and fetal placenta and ear tissues of neonatal calves born from the above cows were collected in 30 min postpartum. All cows were examined and did not show any clinical symptoms, especially any symptoms of ovarian, uterine and mammary diseases.

Preparation of PBMCs and fetal tissues

PBMCs were isolated in one hour after collection. Four milliliters of erythrocyte lysate (8.30 g/l NH_4Cl, 2.0594 g/l Tris and 1,000 ml double-distilled water (ddH_2O) with autoclaving; pH 7.2) was added to 1 ml anticoagulated blood homogenized for 10–15 min and then centrifuged at 3,000 r/min for 10 min. The above manipulation was repeated trice till sediments appeared without erythrocytes. Sediments were mixed with 2 ml phosphate buffered solution (8.00 g/l NaCl, 0.20 g/l KCl, 1.44 g/l Na_2HPO_4, 0.24 g/l KH_2PO_4 and 1,000 ml ddH_2O containing diethyl pyrocarbonate, with autoclaving; pH 7.4) and centrifuged again at 8000 r/min for 40 sec [25]. The final sediments were PBMCs. PBMCs were suspended with phosphate buffered solution (pH 7.4) and adjusted to about 8.0×10^9/l.

Ear tissues were collected from the inferior border of the neonatal calf ear in 30 min postpartum (environment temperature 0–10 C, winter in Shanghai). Placenta and ear tissues were respectively sliced into 5 mm cubes and immediately submersed into liquid nitrogen. PBMCs were mixed with TRIzol soon after isolation (Invitrogen, Carlsbad, CA, USA), snap-frozen in liquid nitrogen immediately, and then transported to the back to lab and stored at –80 C for use in RNA isolation [26, 27].

MHC-I genes

Using the DNAMAN software, 1334 gene hits shown in GenBank were extensively matched and evaluated, and nine pairs of *MHC-I* genes (three non-classical genes and six classical genes containing a heavy chain gene) according to their homology and possible function were chosen for quantitative transcription detection in PBMCs of dairy cows and in fetal ear tissues and the placenta. The nine genes were as follows:

MICB: Bos taurus MHC class I polypeptide-related chain B (*MIC, MICB*), mRNA. NCBI reference sequence: NM_001127317.1 (non-classical)

*BoLA-NC1**: Bos taurus non-classical MHC class I antigen, mRNA (cDNA clone MGC:134252, IMAGE:8064339), complete cds. NCBI reference sequence: BC109706. Synonyms: *BoLA-NC1** (non-classical)

*BoLA-NC3**: Bos taurus haplotype AH11 non-classical MHC class I antigen (BoLA) mRNA, *BoLA-NC3*50201* allele, partial cds. NCBI reference sequence: DQ140378 (non-classical)

Heavy chain gene: Bos taurus MHC class I heavy chain (BoLA), transcript variant 1, mRNA. NCBI Reference Sequence: NM_001038518. Synonyms: BoLA class I histocompatibility antigen, alpha chain BL3-7, D18.5, MGC134453 (classical)

BoLA-A: Bos taurus major histocompatibility complex, class I, A (*BoLA-A*), mRNA. NCBI reference sequence: NM_001114855.1: Synonyms: HD15, MGC142969 (classical)

*BoLA-N*03101*: Bos taurus haplotype AH13 classical MHC class I antigen (BoLA), mRNA. *BoLA-N*03101* allele, partial cds. NCBI reference sequence: DQ140365. Synonyms: A13, AH13-A (classical)

*BoLA-N*03701*: Bos taurus MHC class I antigen (BoLA), mRNA. *BoLA-N*03701* allele, partial cds. NCBI reference sequence: DQ190938 (classical)

*BoLA-N*01201*: Bos taurus MHC class I antigen (BoLA-N), mRNA. *BoLA-N*01201* allele, partial cds. NCBI reference sequence: DQ304655 (classical)

A11: Bos taurus MHC class I antigen (LOC790811), mRNA. NCBI reference sequence: NM_001143743. (A11) Predicted: Bos taurus similar to HLA class I histocompatibility antigen, A-11 alpha chain precursor (MHC class I antigen A*11) (LOC790811), partial mRNA. NCBI reference sequence: XM_001790449.1 (classical).

RNA isolation and reverse transcription

Maternal PBMCs, fetal ear tissues and placentas were schizolysed with cell lysate, and RNA was extracted according to the instructions of an RNApure Reagent Kit (RP1202, BioTeke, Beijing, China). 16 µl RNA solution was diluted to 600 µl and analyzed by ultraviolet spectroscopy (Beckman, DU640). While the photoabsorption at A260/A230 was 2.0, 4 µg/6 µl of RNA was quantified for use in all cases. RNA (5 µl) was confirmed on 1% agarose gel at 300 V for 8 min.

RNA from all samples was treated with RNase free DNase and SuperScript III reverse transcriptase (BioTeke super RT Kit, PR6601) with Oligo (dT) primers for reverse transcription. Six microliters of RNA was mixed with 1 µl Oligo (dT) Primers, 2 µl dNTP Mixture (82650G6, Toyobo, Osaka, Japan) and 5 µl RNase Free ddH_2O and subjected to PCR (Veriti 96-Well Fast Thermal Cycler, Model 9902,Applied Biosystems,Foster City, CA, USA) at 65 C for 5 min and then quenched for 2 min on ice. The above solution was mixed with 1 µl reverse transcriptase M-MULV (200 U/µl; 0003357, Fermentas, Glen Burnie, MD, USA), 4 µl 5× first strand buffer and 1 µl RNase inhibitor and subjected to PCR at 30 C for 10 min, 42 C for 60 min and 95 C for 5 min. The final product was cDNA.

The forward and reverse primers of a cattle housekeeping gene (*Bos Taurus beta actin*, *ACTB*) (Table 1) were used to evaluate the effect of RNA isolation and reverse transcription. The reaction system for *Bos Taurus beta actin* was 0.5 µl Taq (5 µl), 5 µl Buffer, 1 µl dNTP (10 mM), 2 µl cDNA, 2 µl primer F (10 µM), 2 µl primer R (10 µM) and 37.5 µl ddH_2O. The above solution was subjected to PCR with 35 cycles at 95 C for 5 min, 95 C for 30 sec, 55 C for 30 sec, 72 C for 30 sec and 70 C for 10 min (Model 9902,Applied Biosystems). cDNA was confirmed by electrophoresis on 1% agarose

Table 1. Primers of *MHC-I* genes

Genes	Forward primer	Reverse primer	Size of objective segment	Reannealing temperature (C)
MICB	5'-AGAAAGGAGGCTTACATTCCC-3'	5'-GCCTGGTAATGCTTGCTTAAC-3'	199 bp	55
*BOLA-NC1**	5'-AGTATTGGGATCAAGAGACGC-3'	5'-ATAGGCGTGCTGATTATACCC-3'	181 bp	55
*BoLA-NC3**	5'-AGATGACACGAGATGCCAAG-3'	5'-TCGTTCAGGGCGATGTAA-3'	198 bp	55
Heavy chain	5'-TATGTGGACGACACGCAGT-3'	5'-TCGCTCTGGTTGTAGTAGCC-3'	187 bp	55
BoLA-A	5'-GGAGACGCAGAGAACTAAGGA-3'	5'-TCGTTCAGGGCGATGTAA-3'	194 bp	55
*BoLA-N*03101*	5'-GATGACGAGACGCGAATCT-3'	5'-GCGATGTAATCTCTGCCGT-3'	193 bp	55
*BoLA-N*03701*	5'-GTATTGGGATCGGAACACG-3'	5'-AGGTAATCTCTGCCGTCGTAG-3'	171 bp	55
*BoLA-N*01201*	5'-GGAGACGCGAAACTTCAAG-3'	5'-TCGTTCAGGGCGATGTAA-3'	197 bp	55
A11	5'-GAGTATTGGGATGAGGAAACG-3'	5'-AGGTAATCTCTGCCGTCGTAG-3'	199 bp	54
ACTB	5'-TGGACTTCGAGCAGGAGAT-3'	5'-CGTCACACTTCATGATGGAA-3'	194 bp	55

The internal reference gene was *Bos taurus* actin, beta (ACTB); MICB, BOLA-NC1* and BoLA-NC3* are non-classical BoLA-I genes, and the other genes are classical BoLA-I genes.

gel at 300 V for 8 min.

RNA in fetal ear tissues and placentas was isolated with RNALater (Ambion, Austin, TX, USA).

MHC-I sequencing and sequence analysis

The forward and reverse primers of all nine *MHC-I* genes were designed with DNAMAN software according to the *MHC-I* sequences in GenBank (Table 1), and the objective genes were amplified by PCR using the same reaction system and conditions as described before except for use of a different annealing temperature for A11 (54 C).

The amplified segment was separated by electrophoresis on 1% agarose gel at 300 V for 8 min. The objective segment was cut and recovered with a Gel Purification Kit (Spin column) (DP1601, BioTeke). The recovered gene segment was connected with pMD-18-T Vector (TAKARA) and transferred into DH5α competent cells. DH5α cells were cultured at 37 C for 12 h. *MHC-I* segment was amplified from DH5α cell with the primers of the objective gene using the above condition. Amplification of 0.3 μl Taq (5 μ/μl), 2 μl Buffer, 0.3 μl dNTP (10 mM), 1 μl DH5α suspension,1 μl primer F(10 μM),1 μl primer R (10 μM) and 14.4 μl ddH_2O was performed with the conditions described previously. Five microliters of the amplified solution was separated on 1% agarose gel at 300 V for 8 min. All amplified products were sequenced and compared with genes in NCBI BLAST, and the product sizes in agarose gel were similar to objective gene size.

Real-time quantitative PCR analysis of MHC-I transcription

cDNA was diluted 10 times, and the amplification efficiency was validated with *ACTB*. The reaction was performed using 12.5 μl SYBR Green Real-Time PCR Master Mix (QuantiTect SYBR Green PCR Kit, Invitrogen), 0.2 μl primer F (10 μM) (*ACTB* and objective gene), 0.2 μl primer R (10 μM) (*ACTB* and objective gene), 2 μl cDNA and 10.1 μl ddH_2O. The conditions of all objective genes and the candidate reference gene were described before, and quantitative transcription levels were analyzed with real-time quantitative PCR (Bioer FQD-48A, Hangzhou, China).

Statistics

The data from real-time quantitative PCR were analyzed by the Cycle threshold (Ct) method [28]. The level of *MHC-I* transcription ($2^{-\Delta\Delta CT}$) in PBMCs of replacement heifers was set as 1.000, and data from other samples were normalized separately according to it. Subsequently, corrected data from *MHC-I* transcription quantization in different groups were compared using the Student's *t*-test with the SPSS13.0 software.

Results

RNA isolation, cDNA reversely transcription and objective gene identification

Figure 1a shows the total RNA isolated from PBMCs collected from a replacement heifer and pregnant cow in the first and second trimester. All 5s RNAs, 18s RNAs and 28s RNAs displayed bright segments, which validated that RNA was successfully isolated from PBMCs.

The above RNA were reversely transcribed and amplified with the internal referent gene *ACTB*. cDNA were verified on agarose gel electrophoresis (Fig. 1b). All cDNA segments were the objective segments. *MHC-I* genes were successfully amplified from transferred pMD18TDH5α (Fig. 1c, *BoLA-N* 03101* allele). The amplified segment from transferred *DH5α* was sequenced and compared, and the concordance rate was 100% with the objective *MHC-I* gene in NCBI BLAST (the accession number GI: 187937198).

Gene transcription in PBMCs of pregnant cows

Three non-classical genes were variedly expressed in PBMCs in pregnant cows. *MICB* was downregulated in the first and second trimesters ($P<0.05$), but recovered to the level in replacement heifers in the last trimester ($P>0.05$). *BoLA-NC1** was upregulated in the first and last trimesters ($P<0.001$), but no difference was found in the second trimester ($P>0.05$). *BoLA-NC3** was upregulated in all trimesters ($P<0.001$) (Table 2).

There was also a discordance in transcription of four classical genes. Heavy chain was downregulated in all three trimesters ($P<0.001$). *BoLA-A, BoLA-N*03701, BoLA-N*01201* and *A11* showed

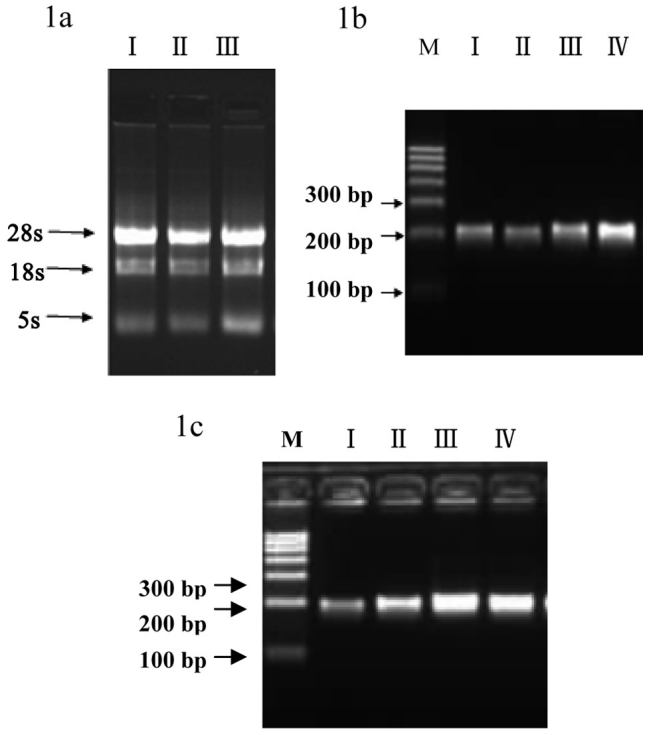

Fig. 1. RNA and cDNA electropherograms, and BoLA-N*03101 allele amplified from pMD18TDH5α. Panel 1a shows RNA isolated from PBMCs in agarose gel electrophoresis. I, II, and III are RNA isolated from PBMCs which collected respectively from a replacement heifer, pregnant cow in the first and second trimester. The bright segments from top to bottom are 28s RNA, 18s RNA and 5s RNA. Panel 1b shows cDNA reverse transcribed and amplified with primers for *Bos taurus* beta actin in 1% agarose gel electrophoresis. The segments from left to right are marker (M), cDNA from a replacement heifer (I), cDNA from a pregnant cow in first (II), second (III) and last (IV) trimester. All of the cDNA segments were at their objective size. Panel 1c shows BoLA-N*03101 allele (IV) amplified from pMD18TDH5α. The segments from left to right are maker (M), BoLA-N*03101 from a replacement heifer (I) and BoLA-N*03101 from a pregnant cow in first (II), second (III) and last (IV) trimesters. All of the cDNA segments were at their objective size.

upregulated expression in all three trimesters, and *BoLA-A* showed the highest level of upregulation in the second trimester (P<0.001), *BoLA-N*03701* showed the highest level of upregulation in the first trimester (P<0.001). *BoLA-N*03101* was downregulated in the first and second trimesters, but upregulated in the last trimester (P<0.001) (Table 2).

Gene transcription in fetal ear tissues and the placenta

The non-classical gene *MICB* was upregulated in fetal ear tissues (P<0.001), and the *BoLA-NC1** gene was almost silent in both fetal placenta and ear tissues (P<0.001); however, the *BoLA-NC3** gene was upregulated in both fetal placenta and ear tissues (P<0.001) (Table 2).

With the exception of the downregulation of heavy chain gene in both fetal placenta and ear tissues (P<0.001), all other classical genes showed their highest levels of upregulation in fetal ear tissues, but were downregulated in the fetal placenta (P<0.001) (Table 2).

Discussion

Non-classical MHC-I transcription in PBMCs of pregnant cows and in fetal tissues

In humans, trophoblast cells express the non-classical MHC class I isotypes HLA-E and HLA-G, which are assumed to prevent fetus destruction by maternal NK cells, which otherwise destroy cells that do not express any MHC class I [29]. HLA-G is expressed on the cell surface of extravillous cytotrophoblast cells from the first trimester placenta, suggesting that it may play a role in the maternal tolerance against the fetal allograft [29]. Usually, fetal alloantigens are exposed to maternal lymphocytes, and then the maternal alloantibody response is stimulated during late pregnancy in cattle [30]. However, maternal cytoimmunity to alloimmunization, although unlikely, could occur, as the relatively noninvasive epitheliochorial placenta in cattle makes leakage of fetal blood cells and trophoblast fragments into the maternal blood impossible [19]. Davies' results showed that besides NC2*, non-classical genes NC3* and NC1* were not expressed in PBMCs in the last trimester (circumnatal period) [13]. These phenomenon, which is not in agreement with with our results, can be explained by differences in the samples used. Davies' PBMC samples were collected from dystocia dams (cesarean delivered) [13], and their results were certainly different from those in eutocia dams. Generally, the most stable stage of pregnancy is the second trimester in all mammal animals. Because it has been rarely reported, we can only presume that upregulated *BoLA-NC1** increased maternal immunity, which was evidenced by it being silenced in fetal tissues and suppressed in the second trimester. *BoLA-NC1** being silenced in fetal tissues could explain why the low immunity resides in the neonatal calf. *BoLA-NC3** inhibits maternal immune response to the fetus, and also inhibits fetal autoimmunity.

The mode of action of the MICB gene is different from other MHC-I genes. MIC is a non-classical MHC-I [23, 31, 32] and has three genotypes, *MICA, MICB* and a pseudogene [33, 34]. Because of its broad similarity to human *MIC* in terms of genomic location, gene structure and limited expression profile, cattle MIC may play an equivalent role to human MIC [31, 35, 36]. Moreover, the structural and functional properties of MICB are similar to those of MICA [37]. As the ligand of nature killer cell receptor G2D (NKG2D), MIC activates NK cells to eliminate pathogen-infected (or tumor) cells, while preventing the killing of normal cells [38]. Although MIC has an important effect on semiallogenic immune response or protection during gestation in humans [35], there is currently no functional data relating to cattle [35]. An implanted exogenous embryo can easily be immunologically rejected by the maternal immune response, and soluble MICB, which was released by placental explants, was present at an elevated level in maternal blood throughout normal pregnancy [39]. These mean that the normal placenta could reduce MIC expression, but apoptosis of the placenta halts the reduction. Actually, the levels of *MICB* were decreased in PBMCs in the first and second trimesters, and recovered to the replacement heifer level in the last trimester in this test. On the fetal side, *MICB* showed its highest level of upregulation in ear tissues and was downregualted

Table 2. *MHC-I* quantitative transcription in PBMCs of pregnant dairy cows, fetal ear tissues and the placenta

Pregnant stage	N	Replacement heifers (PBMCs)	Pregnant cows (PBMCs)			Fetus tissues	
			First trimester	Second trimester	Antepartum	Fetal placenta	Neonatal calf tissues
MICB	10	1 ± 0.019^{a}	0.249 ± 0.029^{B}	0.182 ± 0.011^{B}	0.804 ± 0.043^{a}	0.140 ± 0.012^{B}	309.707 ± 31.639^{C}
*BOLA-NC1**	10	1 ± 0.081^{a}	15.145 ± 0.757^{B}	1.168 ± 0.779^{aC}	3.798 ± 0.178^{D}	0.039 ± 0.003^{E}	0.0185 ± 0.023^{F}
*BoLA-NC3**	10	1 ± 0.045^{a}	14.493 ± 1.019^{B}	9.764 ± 0.674^{C}	12.100 ± 0.434^{B}	2.548 ± 0.236^{E}	64.647 ± 7.716^{F}
Heavy chain	10	1 ± 0.036^{a}	0.111 ± 0.014^{B}	0.107 ± 0.055^{B}	0.104 ± 0.059^{B}	0.007 ± 0.001^{C}	0.393 ± 0.057^{D}
BoLA-A	10	1 ± 0.039^{a}	3.895 ± 0.185^{B}	21.473 ± 1.823^{C}	4.204 ± 0.150^{B}	0.152 ± 0.016^{D}	140.987 ± 15.422^{E}
*BoLA-N*03101*	10	1 ± 0.032^{a}	0.534 ± 0.026^{B}	0.499 ± 0.038^{B}	5.109 ± 0.397^{C}	0.501 ± 0.038^{B}	183.512 ± 11.059^{D}
*BoLA-N*03701*	10	1 ± 0.020^{a}	514.636 ± 23.328^{B}	4.967 ± 0.616^{C}	2.312 ± 0.192^{cb}	0.335 ± 0.0455^{D}	63.765 ± 8.741^{E}
*BoLA-N*01201*	10	1 ± 0.053^{a}	2.062 ± 0.283^{b}	3.132 ± 0.239^{C}	3.403 ± 0.371^{C}	0.153 ± 0.016^{D}	25.757 ± 3.022^{E}
A11	10	1 ± 0.057^{a}	5.347 ± 0.241^{B}	3.421 ± 0.212^{Cb}	3.264 ± 0.171^{Cb}	0.051 ± 0.005^{D}	120.327 ± 16.674^{E}

Different uppercase letters indicate significant differences at $P<0.01$, and the different lowercase letters indicate significant differences at $P<0.05$.

in the fetal placenta. The novel upregulated expression of *MICB* in tissues was favorable for the fetus to escape autoimmunity. On the other hand, the downregulated expression in the fetal placenta enabled it to decohere from the maternal placentome, which was beneficial to delivery [40].

Classical MHC-I transcription in PBMCs of pregnant cows and in fetal tissues

Although classical I gene transcription in the dam was complicated, their transcription regularity was valuable for understanding the mechanism of pregnancy establishment, maintenance and delivery. Human placental trophoblast cells do not express the classical MHC class I isotypes HLA-A and HLA-B, and this absence is assumed to prevent fetus destruction by maternal cytotoxic T cells. Moreover, although transcribed, classical HLA classIgenes are never expressed as membrane-bound products in any of the trophoblast cell subpopulations [29]. However, the transcriptions of *MHC-I* in cattle and dogs were different from those in humans [13]. *MHC-I* mRNA was detected with quantitative RT-PCR and immunohistochemistry, and significant changes were detected after placentation in canine uterine tissues [5]. Similarly, in pregnant bovines, it has been reported that MHC class I expression cannot be detected in the placenta except in late pregnancy under normal conditions and on the maternal caruncular septa of placentomes only, not on fetal cotyledonary villi [41]. Moreover, classical MHC expression is polymorphic; it may also improve abortion and may improve the efficiency of postpartum placental dehiscence in cattle [18, 42, 43].

Presently, no report details the function of bovine classical *MHC-I*. As the ligand for inhibitory receptors on NK cells and other leukocytes, abundant expression of the classical class I proteins represses cytoimmunity to the expressed tissues itself. On the other hand, classical proteins are not excreted into ectoplasm like non-classical proteins [7–9]. The differential transcriptions of classical genes *BoLA-A, BoLA-N*03701, BoLA-N*01201* and *A11* probably represent synergy with the non-classical gene *BoLA-NC3**, which might act as an immune protective mechanism for the pregnant cow itself because all of these genes were upregulated throughout all trimesters if they were further translated as classical MHC-I antigens. *BoLA-N*03101* was downregulated in the first and second trimesters but upregulated in the last trimester, suggesting an initial contribution to pregnancy establishment and maintenance and a contribution to fetus expulsion antepartum. All the classical genes beside heavy chain showed their highest levels of upregulation in neonatal ear tissues, suggesting the protection for fetus itself. However, the contribution of these classical genes to the dam and fetus should be discussed with heavy chain transcription.

MHC-I molecules are heterotrimeric complexes composed of a heavy chain, beta 2 microglobulin (beta2m) and short peptide. This trimeric complex is generated in the endoplasmic reticulum, where a peptide loading complex facilitates transport from the cytosol and binding of the peptide to the preassembled endoplasmic reticulum resident heavy chain/beta2m dimers [44]. MHC class I molecules are composed of a single membrane-spanning heavy chain (including α1, α2 and α3 domains) paired with the soluble protein β2 microglobulin (β2m), which construct a groove in their membrane distal domain (α1α2 or α1β2) that binds peptides in order to display them to the T cells of the cellular immune response [45]. *MHC-I* heavy chain RNA could be downregulated by bovine papillomavirus E5 oncoproteins and bovine herpes virus [46, 47]. A bovine B cell line infected by bovine leukemia virus (BL3.1) is distinguished by a loss of expression of surface MHC-I, which correlates with a diminution in MHC-I heavy chain transcription due to loss of a transactivator [48]. The human cytomegalovirus protein US11 induces dislocation of MHC class I heavy chains from the endoplasmic reticulum (ER) into the cytosol for degradation by the proteasome [49]. Practically, the heavy chain was not detected in PBMCs and binucleate cell lysates by incorporation of radiolabelled methionine, as it did not contain any methionine residues [19]. Classical HLA class I genes could be transcribed, but never expressed as membrane-bound products in any trophoblast cell subpopulations [29]. Trophoblastic cells did not unexpress the classical MHC class I isotypes HLA-A and HLA-B and are assumed to prevent fetus destruction by maternal cytotoxic T cells [29]. Although classical *MHC-II* genes were abundantly expressed, heavy chain gene was significantly downregulated in all dam and fetal tissues compared with the tissues in replacement heifers in this test, suggesting that the effects of classical MHC-I genes are restricted.

In summary, the *BoLA-I* gene composition and overall transcription level in PBMCs were not similar to those in fetal placenta and ear tissues, and their effect on cattle pregnancy was also different.

Non-classical gene *BoLA-NC1** increased maternal immunity to the fetus, but this effect was inhibited by gene *BoLA-NC3**. *BoLA-NC3** also inhibited fetal autoimmunity. Apoptosis of the fetal placenta could reduce *MIC* expression, and upregulated expression of *MICB* in ear tissues was favorable for the fetus to escape autoimmunity. On other hand, downregulated expression of *MICB* in the fetal placenta allowed the placenta to decohere from the maternal placentome, which was beneficial to delivery. Although classical *MHC-I* genes were abundantly expressed, the silence of heavy chain gene restricted their effects.

Acknowledgments

This study was supported by the China National Natural Foundation (No. 30871895 and 31072188). The authors would like to thank Guangming Milk Products Cooperation Ltd. for their contribution to the experiment.

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