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Supplementary Material

MicroRNA-regulated molecular mechanism underlying bovine subclinical endometritis

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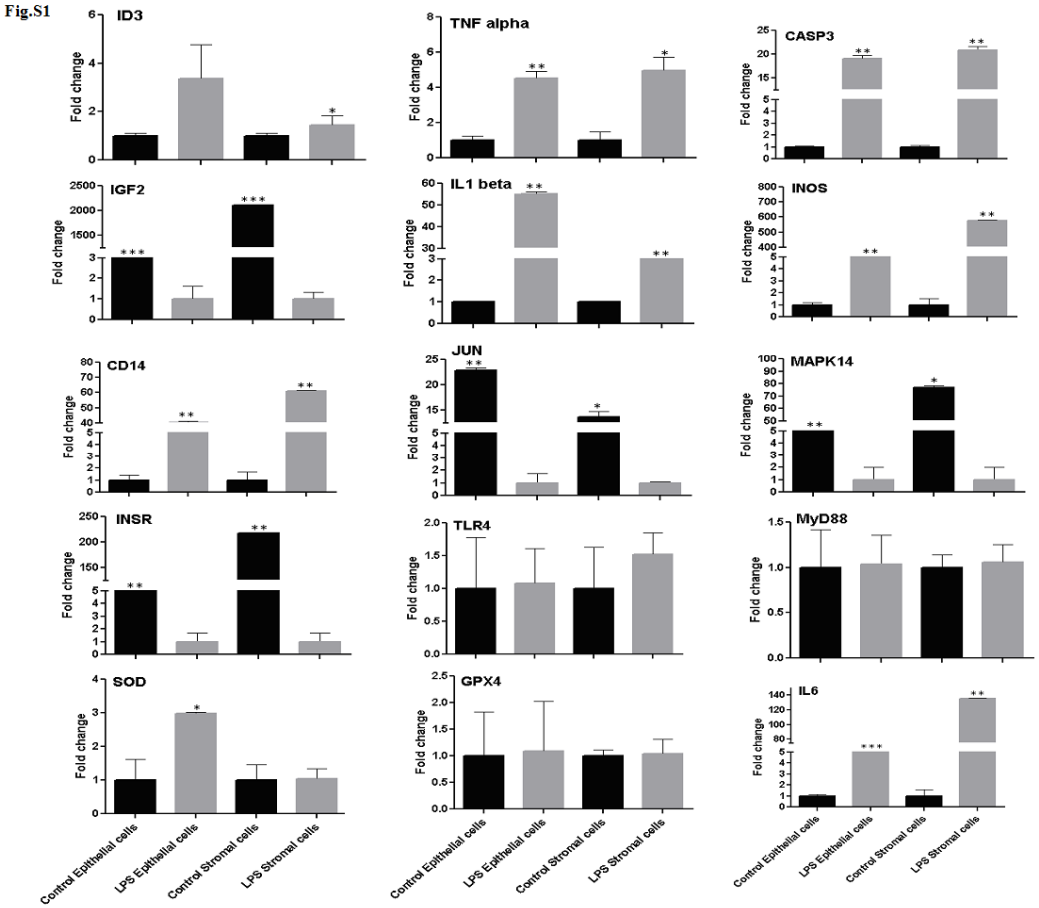

Fig. S1. The expression analysis of selected target genes for the aberrantly expressed microRNAs in *in vitro* cultured endometrial cells challenged by LPS. The analysis was performed using qRT-PCR and statistical significance between control and challenged group are indicated by stars (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$).

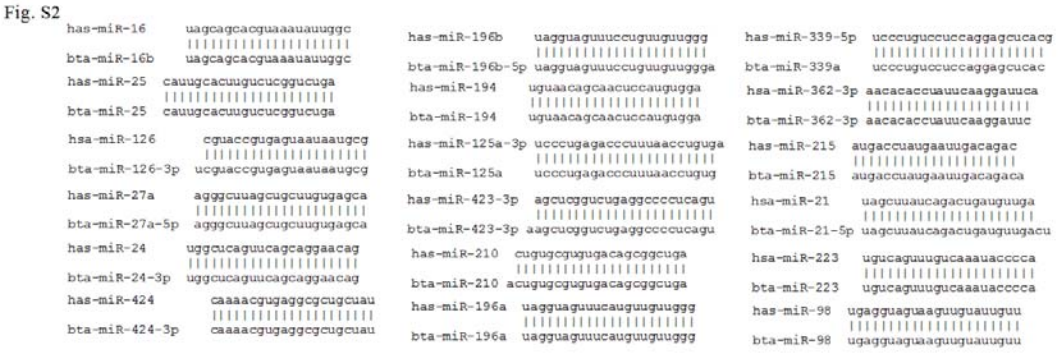

Fig. S2. Alignment of differentially expressed miRNAs in cows with subclinical endometritis with their human ortholog sequences showing sequence conservation of microRNAs between human and bovine.

Table S1. List of primers that were used for PCR amplification of target genes

Gene name	Primer sequence (5′→3′)	Annealing temperature (°C)
- *GPX4*	F -ACCCTCTGTGGAAATGGATG R -GAAGGCTTCTCGGAACACAG	55
- *TGFβ1*	F -CACGTGGAGCTGTACCAGAA R -GCGAAAGCCCTCTATTTCCT	55
- *IGF1*	F -TTGCACTTCAGAAGCAATGG R -ACTGGAGAGCATCCACCAAC	54
- *INOS*	F -TGTTCAGCTGTGCCTTCAAC R -AAAGCGCAGAACTGAGGGTA	55
- *CASP3*	F -TGCCACTGTATCAGGGAACA R -TGCTCAGCACAAACATCACA	52
- *IL6*	F -TGCAGTCTTCAAACGAGTGG R -TAAGTTGTGTGCCCAGTGGA	54
- *CD 14*	F -TATCGTGGACAACAGGAGGT R -GCGTAGCGCTAGATATTGGA	54
- *TLR4*	F -AGAGCCACTTCTGGTCACAG R -TAAAGCTCAGGTCCAGCATC	55
- *MYD88*	F -CCTCTCATCTGCCTTTTTGA R -GCCCCAGAAAGAAAGACTTC	53
- *CD45*	F -CAAAGAGCCCAGGAAGTACA R -GTTGATCTCCACAATCACA	51
-*IL1β*	F -CCTTGGGTATCAAGGACAAG R -CGATTTGAGAAGTGCTGATG	53
- *SOD*	F -CCTTGGGTATCAAGGACAAG R -CGATTTGAGAAGTGCTGATG	50
- *TNF-α*	F -CTTCCACCCCCTTGTTCCT R -AGGCGATCTCCCTTCTCCA	55

Table S2. List of primers used to amplify binding sites of miRNAs in the 3'UTR of target genes

TD, touchdown PCR

Gene name	Primer sequence (A)	Annealing temperature (°C) (A)
MAPK14	F -CTAGTGTGGGAGGGTAAAACATGATG R-TCGACGTCTACTCCGGATTCACACG	60
NOS2	F - CTAGGGAACTTAATGATGGCACCCA R - TCGAGAGTGGCGTGACAGCGC	TD: 62–59
JUN	F- CTAGCAAGTTGCGAGAGGGAAACAG R -TCGAATCCTTTCTGGAATTTTCAGAAACA	TD: 61–57
IKBKB	F- CTAGACCCCTCCTGCTCTCCAAAG R- TCGACCGTGCTGTCCTTCTGTTACAC	62
INSR	F - CTAGGTGGCCTTTTGCTTGGTCTTC R - TCGACCTTAGCGGTTTCTCCTCTGG	61
SP1	F - CTAGGCTGGGAGGAGGAAGGAGAC R- TCGATGGAGATGTTCAAAAGAGGAAGTG	TD: 64-60
ID3	F - CTAGCCTCCAGAACGCAGGTGC R - TCGACATCGCATGGTTACAGAAAGTCAC	61

Table S3. Sense and antisense oligo nucleotide sequences with three nucleotide mismatches in the miRNA target site

miRNA target mismatch	Nucleotide sequence
miR-423-3p	Sense 5'-CTAGCTAGCGGCCGC TAGTACTGAGGGGCCTCAGA**GGC**GCTG-3'
	Antisense 5'-TCGACAGC**GCC**TCTGAGGCCCCTCAGTACTAGCGGCCGCTAG-3'
miR-196b	Sense 5´-CTAGCTAGCGGCCGCTAGTCCCAACAACAGGAAACT**TGG**AG-3'
	Antisense 5'- TCGACT**CCA**AGTTTCCTGTTGTTGGGACTAGCGGCCGCTAG-3'
miR-24	Sense 5' – CTAGCTAGCGGCCGCTAGTCTGTTCCTGCTGAACTG**CGG**AG-3'
	Antisense 5' – TCGACT**CCG**CAGTTCAGCAGGAACAGACTAGCGGCCGCTAG-3'
miR-503	Sense 5'- CTAGCTAGCGGCCGCTAGTCTGCAGAACTGTTCCCG**G**T**CG**AG-3'
	Antisense 5'- TCGACT**CG**A**C**CGGGAACAGTTCTGCAGACTAGCGGCCGCTAG-3'
miR-27a	Sense 5'- CTAGCTAGCGGCCGCTAGTGCGGAACTTAGCCA**G**T**C**T**C**AG-3'
	Antisense 5'- TCGACT**G**A**G**A**C**TGGCTAAGTTCCGCACTAGCGGCCGCTAG-3'