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## Supplementary Material

### MicroRNA-regulated molecular mechanism underlying bovine subclinical endometritis

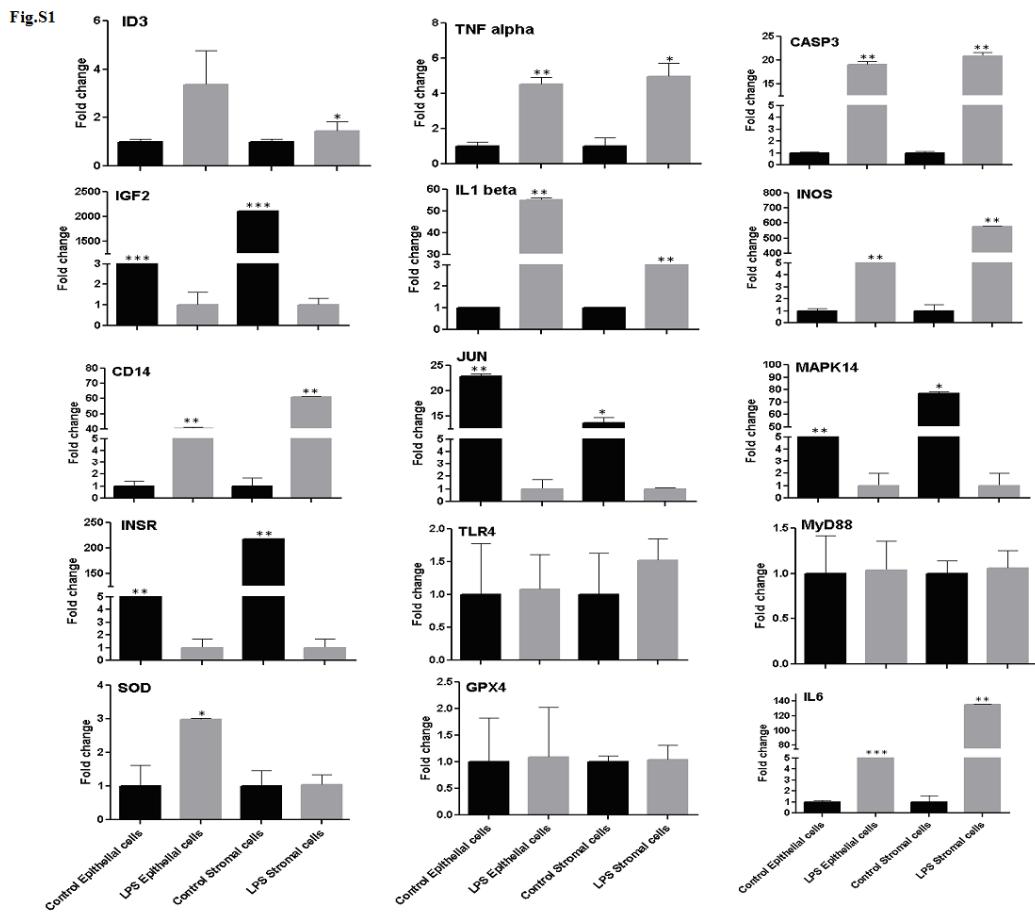
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**Fig. S1.** The expression analysis of selected target genes for the aberrantly expressed microRNAs in *in vitro* cultured endometrial cells challenged by LPS. The analysis was performed using qRT-PCR and statistical significance between control and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

**Fig. S2**

has-miR-16	uagcagcacquaaaaauuggc	has-miR-196b	uagguaquuuuccuguuuggg	has-miR-339-5p	ucccuquuccuccaggagcucacg
bta-miR-16b	uagcagcacquaaaaauuggc	bta-miR-196b-5p	uagguaquuuuccuguuuggg	bta-miR-339a	ucccuquuccuccagjagcuc
has-miR-25	cauugcacuugucggucuga	has-miR-194	uquaacagcaacuccauugga	hsa-miR-362-3p	aacacaccuuucaaggauca
bta-miR-25	cauugcacuugucggucuga	bta-miR-194	uquaacagcaacuccauugga	bta-miR-362-3p	aacacaccuuucaaggauuc
hsa-miR-126	cguaccgugagauaaaaugcg	has-miR-125a-3p	ucccugagacccuuuaccugua	has-miR-215	augaccuuuugaauugacagac
bta-miR-126-3p	ucguaccgugagauaaaaugcg	bta-miR-125a	ucccugagacccuuuaccugug	bta-miR-215	augaccuuuugaauugacagaca
has-miR-27a	agggcuauagcugcuuugagca	has-miR-423-3p	agcuoggucugaggcccucagu	hsa-miR-21	uagcuuauagacugauugua
bta-miR-27a-5p	agggcuauagcugcuuugagca	bta-miR-423-3p	agcuoggucugaggcccucagu	bta-miR-21-5p	uagcuuauagacugauugacu
has-miR-24	uggcucaguucagcggaaacag	has-miR-210	cugugcugugacacgcgcugua	hsa-miR-223	uugcaguuugucaaaacccca
bta-miR-24-3p	uggcucaguucagcggaaacag	bta-miR-210	acugugcugugacacgcgcugua	bta-miR-223	uugcaguuugucaaaacccca
has-miR-424	caaaaacgugaggccugcuau	has-miR-196a	uagguaquuucauguuuggg	has-miR-98	ugagguaquaaguuguauuuuu
bta-miR-424-3p	caaaaacgugaggccugcuau	bta-miR-196a	uagguaquuucauguuuggg	bta-miR-98	ugagguaquaaguuguauuuuu

**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)
- <i>GPX4</i>	F -ACCCTCTGTGGAAATGGATG R -GAAGGCTTCTCGGAACACAG	55
- <i>TGFβ1</i>	F -CACGTGGAGCTGTACCAGAA R -GCGAAAGCCCTCTATTCCT	55
- <i>IGF1</i>	F -TTGCACTTCAGAAGCAATGG R -ACTGGAGAGCATCCACCAAC	54
- <i>INOS</i>	F -TGTCAGCTGTGCCTTCAAC R -AAAGCGCAGAACTGAGGGTA	55
- <i>CASP3</i>	F -TGCCACTGTATCAGGGAAACA R -TGCTCAGCACAAACATCACA	52
- <i>IL6</i>	F -TGCAGTCTTCAAACGAGTGG R -TAAGTTGTGTGCCAGTGGA	54
- <i>CD14</i>	F -TATCGTGGACAACAGGAGGT R -GCGTAGCGCTAGATATTGGA	54
- <i>TLR4</i>	F -AGAGCCACTTCTGGTCACAG R -TAAAGCTCAGGTCCAGCATC	55
- <i>MYD88</i>	F -CCTCTCATCTGCCTTTGA R -GCCCCAGAAAGAAAGACTTC	53
- <i>CD45</i>	F -CAAAGAGCCCAGGAAGTACA R -GTTGATCTCCACAATCACA	51
- <i>IL1β</i>	F -CCTTGGGTATCAAGGACAAG R -CGATTGAGAAGTGCTGATG	53
- <i>SOD</i>	F -CCTTGGGTATCAAGGACAAG R -CGATTGAGAAGTGCTGATG	50
- <i>TNF-α</i>	F -CTTCCACCCCTGTTCCCT R -AGGCGATCTCCCTCTCCA	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)
<i>MAPK14</i>	F -CTAGTGTGGGAGGGTAAACATGATG R-TCGACGTCTACTCCGGATTCACACG	60
<i>NOS2</i>	F - CTAGGGAACTTAACATGATGGCACCCA R - TCGAGAGTGGCGTGACAGCGC	TD: 62–59
<i>JUN</i>	F- CTAGCAAGTTGCGAGAGGGAAACAG R - TCGAACCTTCTGGAATTTCAGAAACA	TD: 61–57
<i>IKBKB</i>	F- CTAGACCCCTCCTGCTCTCCAAAG R- TCGACCGTGCTGTCCTCTGTTACAC	62
<i>INSR</i>	F - CTAGGTGGCCTTTGCTTGGTCTTC R - TCGACCTTAGCGGTTCTCCTCTGG	61
<i>SPI</i>	F - CTAGGCTGGGAGGAGGAAGGAGAC R- TCGATGGAGATGTTAAAAGAGGAAGTG	TD: 64-60
<i>ID3</i>	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 <u>GGG</u> GCTG-3' Antisense 5'-TCGACAGC <u>GCC</u> CTTGAGGCCCTCAGTACTAGCGGCCGCTAG-3'
miR-196b	Sense 5'-CTAGCTAGCGGCCGCTAGTCCAACAAACAGGAA <u>ACTTGGAG</u> -3' Antisense 5'- TCGACT <u>CCAAG</u> TTCCTGTTGTGGACTAGCGGCCGCTAG-3'
miR-24	Sense 5' - CTAGCTAGCGGCCGCTAGTCTGCTGA <u>ACTGCGGAG</u> -3' Antisense 5' - TCGACT <u>CCG</u> CAGTTCAGCAGGAACAGACTAGCGGCCGCTAG-3'
miR-503	Sense 5' - CTAGCTAGCGGCCGCTAGTCTGCAGAA <u>CTGTCGAG</u> -3' Antisense 5' - TCGACT <u>CGACC</u> GGGAACAGTTCTGCAGACTAGCGGCCGCTAG-3'
miR-27a	Sense 5' - CTAGCTAGCGGCCGCTAGTGC <u>GGAACTTAGCCAGTCTCAG</u> -3' Antisense 5' - TCGACT <u>GA</u> ACTGGCTAAGTCCGCACTAGCGGCCGCTAG-3'