

## **GEBREMEDHN ET AL., SUPPLEMENTAL FIGURE LEGENDS**

**Supplemental FIGURE S1: Detection of Granulosa and luteinizing cell specific marker genes.** Identity of granulosa cells and possible transformation into luteal cells were assessed by semi-quantitative PCR. Granulosa cell-specific marker gene *FSHR* was detected in granulosa cells both before and after plating. The Luteinizing cell marker gene (*LHR*) was not detected in the cells both before and after culture. RNA sample isolated from luteinizing granulosa cells derived from preovulatory dominant follicle on day 19 of estrous cycle was used as positive control. Genomic DNA (gDNA) was used as PCR positive control (**A**). *In vitro* culture decrease the expression of LH induced ovulatory genes (*PTX3*; **B**) and (*PTGS2*; **C**) signifying the absence luteinization (**B**).

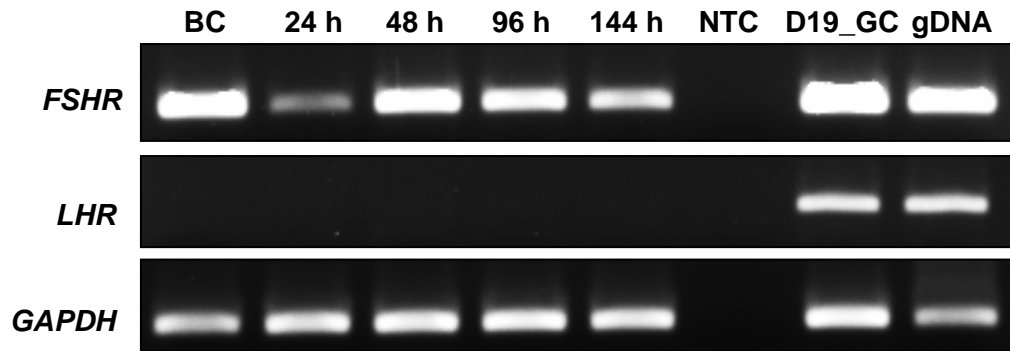
**Supplemental FIGURE S2: Genomic organization and sequence conservation of bovine miR-183~96~182 cluster miRNAs.** MiR-183~96~182 cluster is transcribed from intergenic region of chromosome 4 of bovine genome. Members of the miRNA cluster have higher sequence homology with similar seed region (**A**). Members of the miRNAs cluster are evolutionarily conserved across different mammalian species. bta, *Bos taurus*; has, *Homo sapiens*; mmu, *Mus musculus*; rno, *Rattus norvegicus* (**B**).

**Supplemental FIGURE S3: FASL is not a predicted target gene of miR-183~96~182 cluster miRNAs.** MiRNA target prediction using target scan 6.0 [55-57] showed that the 3'-UTR of bovine *FASL* harbors the binding sites of several miRNAs. However, there are no conserved binding sites for the miR-183~96~182 cluster miRNAs.

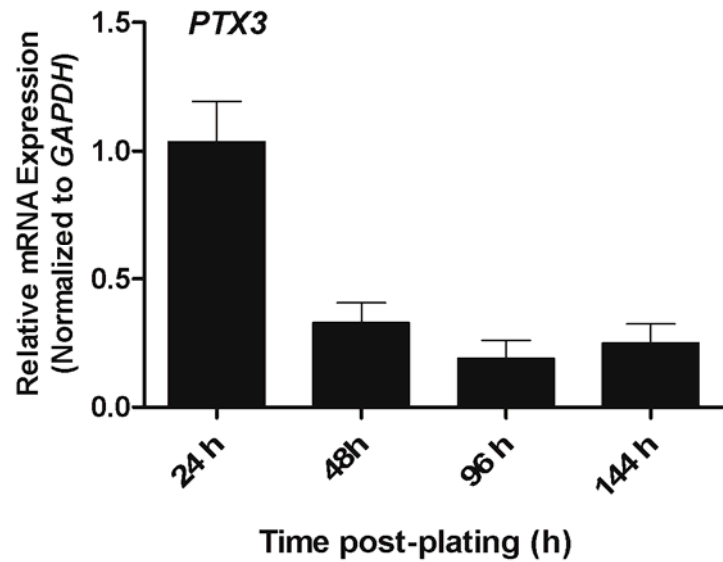
## **REFERENCES USED IN SUPPLEMENTAL DATA**

55. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*. 2005 Jan 14;120(1):15-20.
56. Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol Cell* 2007; 27: 91-105.
57. Friedman RC, Farh KK, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res* 2009; 19: 92-105.

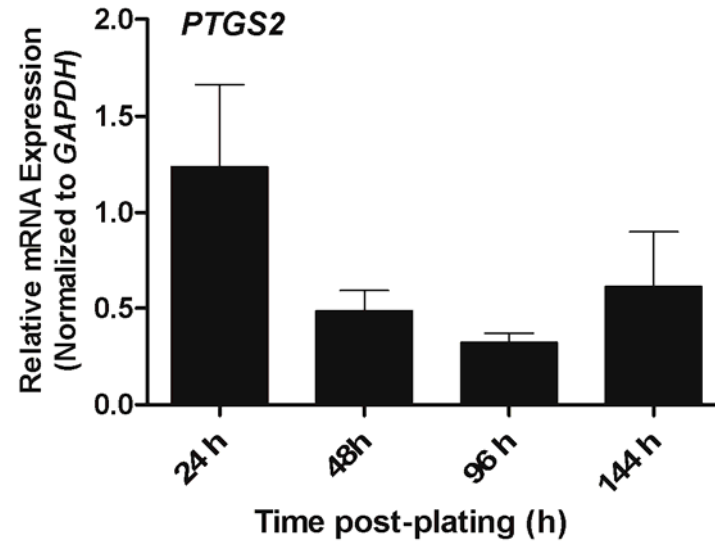
A



B



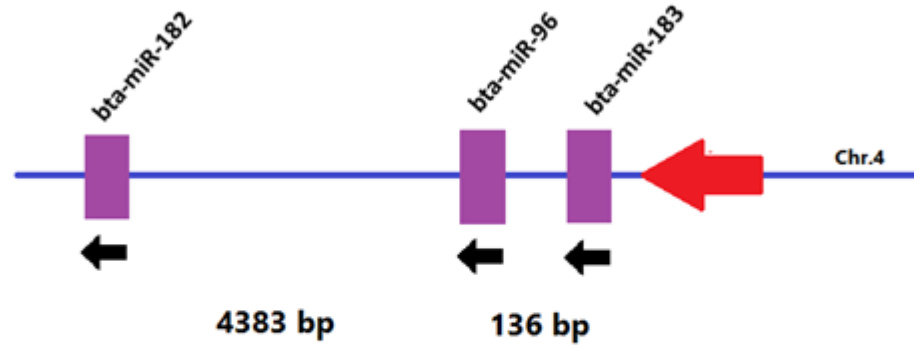
C



Gebremedhn et al., Supplemental Figure S1

A

Gebremedhn et al., Supplemental Figure S2



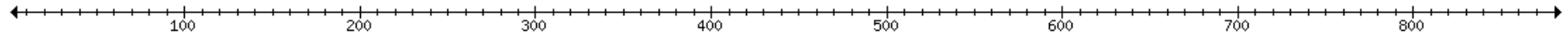
**bta-miR-183** UAUGGCACUGGUAGAAUUCACUG  
**bta-miR-96** UUUGGCACUAGCACAUUUUUGCU  
**bta-miR-182** UUUGGCAAUGGUAGAACUCACACU

B

miR-96	bta	UUUGGCACUAGCACAUUUUUGCU --
	hsa	UUUGGCACUAGCACAUUUUUGCU --
	mmu	UUUGGCACUAGCACAUUUUUGCU --
	rno	UUUGGCACUAGCACAUUUUUGCU --
miR-182	bta	UUUGGCAAUGGUAGAACUCACACU -
	hsa	UUUGGCAAUGGUAGAACUCACACU -
	mmu	UUUGGCAAUGGUAGAACUCACACCG
	rno	UUUGGCAAUGGUAGAACUCACACCG
miR-183	bta	UAUGGCACUGGUAGAAUUCACUG --
	hsa	UAUGGCACUGGUAGAAUUCACU ---
	mmu	UAUGGCACUGGUAGAAUUCACU ---
	rno	UAUGGCACUGGUAGAAUUCACU ---

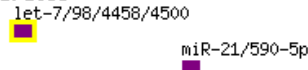


### Cow FASLG 3' UTR



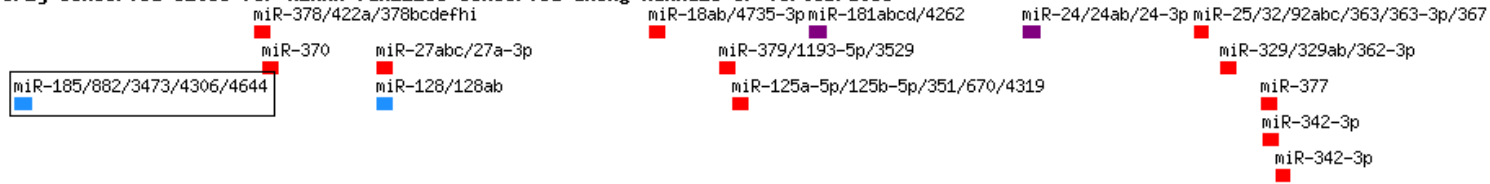
Gene  
Cow FASLG NM\_000639 3' UTR length:883

**Conserved sites for miRNA families broadly conserved among vertebrates**



**Conserved sites for miRNA families conserved only among mammals**

**Poorly conserved sites for miRNA families conserved among mammals or vertebrates**



**Key:**  
 Sites with higher probability of preferential conservation  
 8mer (yellow square) 7mer-m8 (red square) 7mer-1A (blue square) 3' comp\* (green square)  
 Sites with lower probability of preferential conservation  
 8mer (purple square) 7mer-m8 (red square) 7mer-1A (blue square) 3' comp\* (green square)

Gebremedhn et al., Supplemental Figure S3