

## Article

# Potential of Circulating miRNA Biomarkers and Exosomes for Early Pregnancy Diagnosis in Cattle

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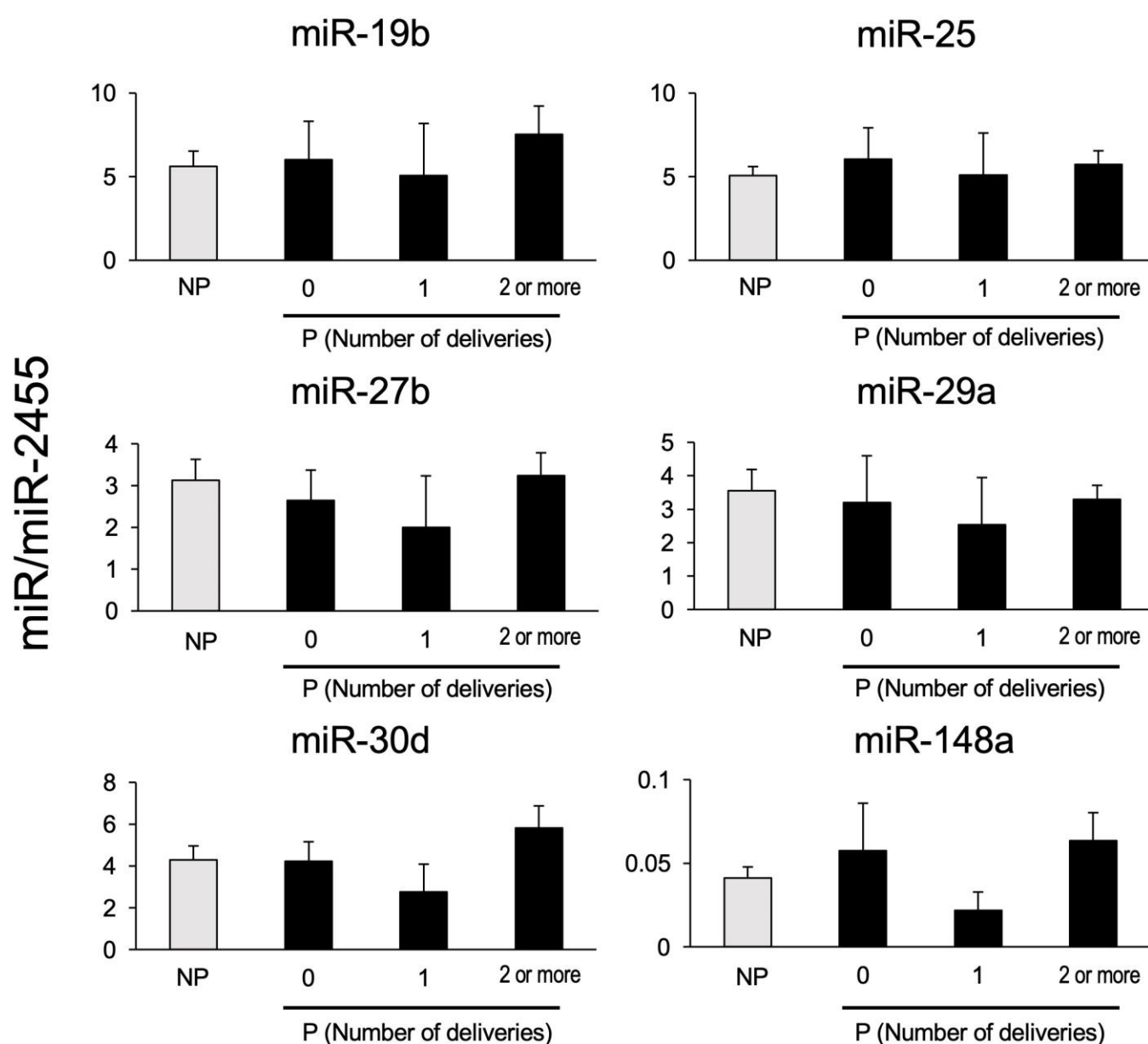
**Table S1.** List of standard oligonucleotide sequences synthesized by RT-qPCR.

miRNA	Sequence (5'-3')	Bases
bta-miR-19b	AGGCAGTGGTATCAACGCAGAG- TACTTTTTTTTTTTTTTTTTTTTTTTTTTTTCAG- TTTGATGGATTGCGA	78
bta-miR-25	AGGCAGTGGTATCAACGCAGAG- TACTTTTTTTTTTTTTTTTTTTTTTTTTTTTCAGAC- CGAGACAAGTGCAATG	77
bta-miR-27b	AGGCAGTGGTATCAACGCAGAG- TACTTTTTTTTTTTTTTTTTTTTTTTTTTTT- GCAGAACTAGCCACTGTGAA	76
bta-miR-29a	AGGCAGTGGTATCAACGCAGAG- TACTTTTTTTTTTTTTTTTTTTTTTTTTTTAAC- CGATTTCAGATGGTGCTAG	78
bta-miR-30d	AGGCAGTGGTATCAACGCAGAG- TACTTTTTTTTTTTTTTTTTTTTTTTTTTTTAGCTTCCAGTCGGG GATGTTTACA	79
bta-miR-126-3p	AGGCAGTGGTATCAACGCAGAG- TACTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGCGTAC- CAAAAGTAATAATG	76
bta-miR-148a	AGGCAGTGGTATCAACGCAGAG- TACTTTTTTTTTTTTTTTTTTTTTTTTTTTTACAAAGTTCTGTAG TGCACTGA	77
bta-miR-2455	AGGCAGTGGTATCAACGCAGAG- TACTTTTTTTTTTTTTTTTTTTTTTTTTTTCCCTGCCTCCCCG AGCACAGA	77

**Table S2** Plasma miRNA and exosome miRNA levels of pregnant and non-pregnant cows on Day 21 after AI.

miRNA	NP/P	Whole plasma miRNA (copy number)	Exosomal miRNA (copy number)	Exosomal miRNA (%)
miR-19b	NP	27,258 ± 343	92 ± 13	0.34
	P	27,697 ± 8355	70 ± 10	0.25
miR-25	NP	37,141 ± 4336	383 ± 44	1.03
	P	43,446 ± 14,506	296 ± 108	0.68
miR-27b	NP	14,415 ± 812	33 ± 10	0.23
	P	17,687 ± 5,744	60 ± 42	0.34
miR-29a	NP	20,178 ± 4,609	120 ± 45	0.60
	P	27,909 ± 8852	79 ± 16	0.28
miR-30d	NP	34,558 ± 10,965	42 ± 7	0.12
	P	39,017 ± 18,397	23 ± 8	0.06
miR-126-3p	NP	12,023 ± 4,174	245 ± 107	2.03
	P	9,043 ± 3,089	83 ± 35	0.91
miR-2455	NP	10,850 ± 1369	258 ± 45	2.38
	P	18,873 ± 19243	324 ± 154	1.72

The miRNA levels in the plasma of pregnant (P, n = 3) and non-pregnant (NP, n = 3) cows on Day 21 after AI were analyzed using RT-qPCR. The levels indicate the copy numbers of 1 ng of each miRNA or small RNA. Data are shown as mean ± SD. The percentage of exosomal miRNAs relative to total plasma miRNAs is also indicated.



**Figure S1.** Circulating miRNA levels in pregnant cows for each reproductive history and in non-pregnant cows on Day 21 after AI. The miRNA levels in the plasma of pregnant (P, n = 22) and non-pregnant (NP, n = 15) cows on Day 21 after AI were analyzed using RT-qPCR and normalized to miR-2455 levels. Pregnant cows were divided according to their reproductive history into heifers (0, n = 5), primiparous (1, n = 4), and multiparous (2 or more, n = 13) cows. Data are shown as the mean  $\pm$  SEM.