Indian Journal of Animal Sciences **86** (10): 1153–1154, October 2016/Short communication

Expression of the Mir-183 cluster in the follicles and corpus luteum of cattle

TENG-HE MA[1], LONG CHENG[2], BIN WANG[3], GUAN-HUI LIU[4], LI-NA XU[5], YING BAI[6] and YU-XIANG SHI[7]

Hebei University of Engineering, Handan 056 021 China

Received: 6 March 2016; Accepted: 5 April 2016

Key words: Cattle, Corpus luteum, Follicle, MiR-183 cluster

MicroRNAs (miRNAs) are small non-coding endogenous RNA molecules and involved in bovine follicle development (Gebremedhn *et al.* 2015) and luteal function (Maalouf *et al.* 2014). Meagre information is available about the functions of miR-183 cluster in cattle reproduction system. Therefore, we studied the expression of the miR-183 cluster in dominant and subordinate bovine follicles and corpus luteum (CL) with different phases.

The ovaries were from estrous synchronized simmental heifers and collected by cutting vagina. The size of each follicle was classified as a dominant or subordinate follicle depending on the diameter as previously recommended (Spicer *et al.* 2011) with minor modifications. In brief, follicles with follicular diameters ≤11 mm were classified as subordinate, whereas those with diameters ≥12 mm were considered dominant. The CL samples were collected according to Miyamoto *et al.* (2000) report. The pellets of follicles and early, mid-, late, or regressed CLs were stored in –80°C for RNA extration.

Total RNA was isolated from follicle pellets and CLs using miRNA isolation kit. The first-strand cDNA was synthesized by qPCR RT kit. Relative quantification of miR-182, miR-96, and miR-183 was performed using SYBR Real-time PCR Master Mix as standard protocol. U6 small nuclear RNA served as endogenous control. The primers of miRNA-specific RT and real-time PCR are given in Table 1.

The relative expression levels of miRNA and U6 were calculated by the comparative threshold cycle method (Livak and Schmittgen 2001) and analyzed by student's *t*-test or one-way ANOVA using SPSS 12 software.

The expression of the miR-183 cluster was detected in all follicles and CLs (Figs 1, 2). For the follicle samples, all members of the miR-183 cluster showed higher expression levels in dominant follicles than in subordinates. Using Illumina miRNA deep sequencing, Gebremedhn reported that miR-183 clusters are significantly enriched with preovulatory dominant follicles (Gebremedhn *et al.* 2015). This result was similar to our findings. The

Table 1. Primers used for Real-time RT-PCR

Primer	Sequence (10 pmol/µl)	Annealing temperature (°C)	Product size (bp)
miR-182	RT: 5'GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCAC TGGATACGACAGTGTG3' F: 5'GGGGTTTGGCAATGGTAGAACT3'	59	66
miR-96	RT: 5'GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCAC TGGATACGACAGCAAAAA3' F: 5'GGGGTTTGGCACTAGCACA3'	59	65
miR-183	RT: 5'GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCAC TGGATACGACCAGTGA3' F: 5'GGGGTATGGCACTGGTAGAAT3'	59	65
Universal U6	R: 5'GTGCGTGTCGTGGAGTCG3' F: 5'GCTTCGGCAGCACATATACTAAAAT3' R(RT): 5'CGCTTCACGAATTTGCGTGTCAT3'	59	89

Present address: [1,3,4,5,6]Scientist (matenghe@126.com, hdwangbin@126.com, liuguanhui.1986@163.com, 51765722 @qq.com, 562625563@qq.com), [7]Principal Scientist (hbyxshi@126.com), Division of Animal Science, [2]Scientist (chenglong8211@163.com), Animal Diseases Control and Prevention of Handan.

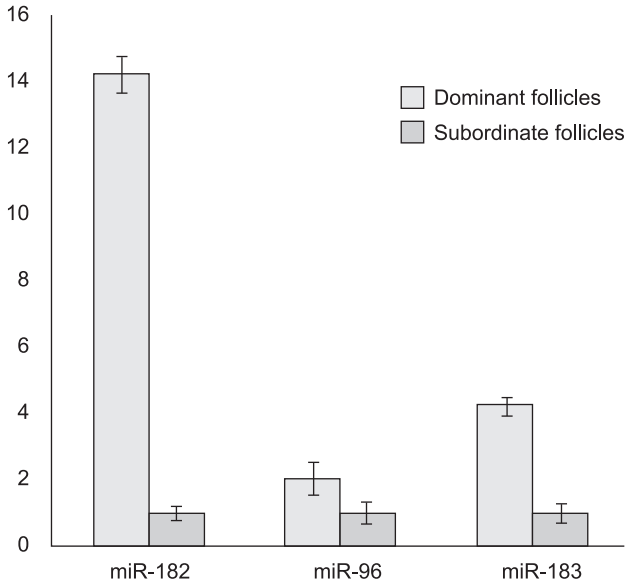

Fig. 1. Relative expression pattern of miR-183, miR-96, and miR-182 in dominant bovine or subordinate bovine follicles (mean±SE). **P<0.01, *P<0.05.

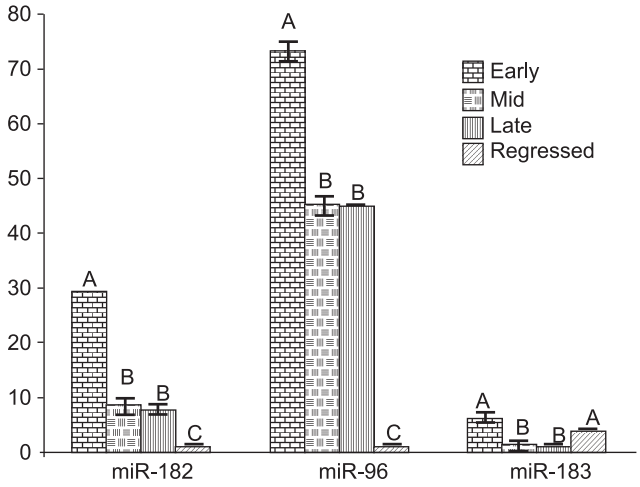

Fig. 2. Relative expression pattern of miR-183, miR-96, and miR-182 in bovine early, mid-, late, and regressed CL (mean±SE). Different shoulder letters differ significantly (P<0.01).

expression levels of miR-182 and miR-183 were significantly upregulated (P<0.01), but the expression of miR-96 showed no significant increase (P>0.05), which indicated miR-182 and miR-183 may play more dominant functions in follicle development.

For the CL samples, miR-96 exhibited an expression pattern similar to miR-182, which showed no significant difference in the mid and late CL phases. Normally, members of a cluster have the same expression characteristics and functions, but our results showed that miR-183 exhibited lower expression levels in the mid and late CL phases than that in early and regressed CL phases. Numerous miRNAs, such as miR-126 (Dai *et al.* 2014) and miR-34a (Maalouf *et al.* 2016), function in bovine CL, but no reports about the function of the miR-183 cluster within bovine CL were found. The miR-183 has a different expression tendency from that of miR-182 and miR-96, indicating that it has a different function in CL development and requires further study.

In conclusion, this study demonstrated that the miR-183 cluster showed different expression patterns in dominant and subordinate bovine follicles or in various luteal phases. Thus, miR-183 cluster may participate in important physiological processes in the bovine ovary.

SUMMARY

In this study, the expression profiles of the miR-183 cluster were explored in bovine dominant and subordinate follicles and different luteal phases. This miRNA cluster showed higher expression levels in dominant bovine follicles than in subordinates. The expression levels of miR-182 and miR-96 were reduced from the early phase to the regressed phase, whereas miR-183 showed lower expression levels in the mid and late CL phases than in the early and regressed CL phases.

ACKNOWLEDGMENT

This study was supported by Youth Foundation of Hebei Education Department (QN2015012), Natural Science Foundation of Hebei Province (C2016402061) and Doctoral Scientific Research Foundation, Hebei University of Engineering (20120139).

REFERENCES

Dai L, Xu J, Liu S, Ma T, Zhu Y, Xu F, Gao Y, Yuan B, Wang S, Zhang Y, Sun G and Zhang J. 2014. Characterization of miR-126–3p and its target talin2 in the bovine corpus luteum during the oestrus cycle. *Reproduction in Domestic Animals* **49**(6): 913–19.

Gebremedhn S, Salilew-Wondim D, Ahmad I, Sahadevan S, Hossain M M, Hoelker M, Rings F, Neuhoff C, Tholen E, Looft C, Schellander K and Tesfaye D. 2015. MicroRNA expression profile in bovine granulosa cells of preovulatory dominant and subordinate follicles during the late follicular phase of the estrous cycle. *PLoS One* **10**(5): e0125912.

Livak K J and Schmittgen T D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* **25** (4): 402–08.

Maalouf S, Smith C and Pate J L. 2016. Changes in microRNA expression during maturation of the bovine corpus luteum: regulation of luteal cell proliferation and function by microRNA-34a. *Biology of Reproduction* **94**(3):71.

Maalouf S W, Liu W S, Albert I and Pate J L. 2014. Regulating life or death: potential role of microRNA in rescue of the corpus luteum. *Molecular and Cellular Endocrinology* **398** (1–2): 78–88.

Miyamoto Y, Skarzynski D J and Okuda K. 2000. Is tumor necrosis factor alpha a trigger for the initiation of endometrial prostaglandin F(2alpha) release at luteolysis in cattle. *Biology of Reproduction* **62** (5): 1109–15.

Spicer L J, Schreiber N B, Lagaly D V, Aad P Y, Douthit L B and Grado-Ahuir J A. 2011. Effect of resistin on granulosa and theca cell function in cattle. *Animal Reproduction Science* **124** (1–2): 19–27.