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Cathepsin D knockdown regulates biological behavior of granulosa cells and affects litter size traits in goats

JZUS-B

Key words: CTSD; Litter size traits; Granulosa cells; Cell apoptosis; Cell cycle; Cell proliferation

1 Introduction

‘Qianbei Ma’ goat is a fine local breed of Guizhou Province in China, with a litter size rate up to 196%. It is considered as an ideal model for studying the reproductive traits of goats.

Cathepsin D (CTSD), which is a member of the lysosomal aspartic protease family. Previous transcriptome sequencing findings showed that, in comparison with monotocous goats, CTSD expression was significantly downregulated in the ovarian tissue of polytocous goats (unpublished data). Therefore, CTSD regulates follicular development and biological function in goat granulosa cells. However, no study has yet evaluated the effect of CTSD on the biological behavior of goat granulosa cells and the litter size trait.

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2 Innovation points

In this work, we

- **(i) explored the correlation between CTSD expression and litter size in goats and the effects of CTSD on the biological behavior of granulosa cells, as well as the location of CTSD in goat ovarian tissue;**
- **(ii) analyzed the differential expression of CTSD in ovarian tissues between monotocous and polytocous 'Qianbei Ma' goats;**
- **(iii) revealed the effects of CTSD knockdown on cell proliferation, apoptosis, cell cycle, and the expressions of candidate genes of the prolific trait in granulosa cells.**

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3 Research Summary

- **Immunohistochemistry**

CTSD was revealed to exist in oocytes from all levels of follicles

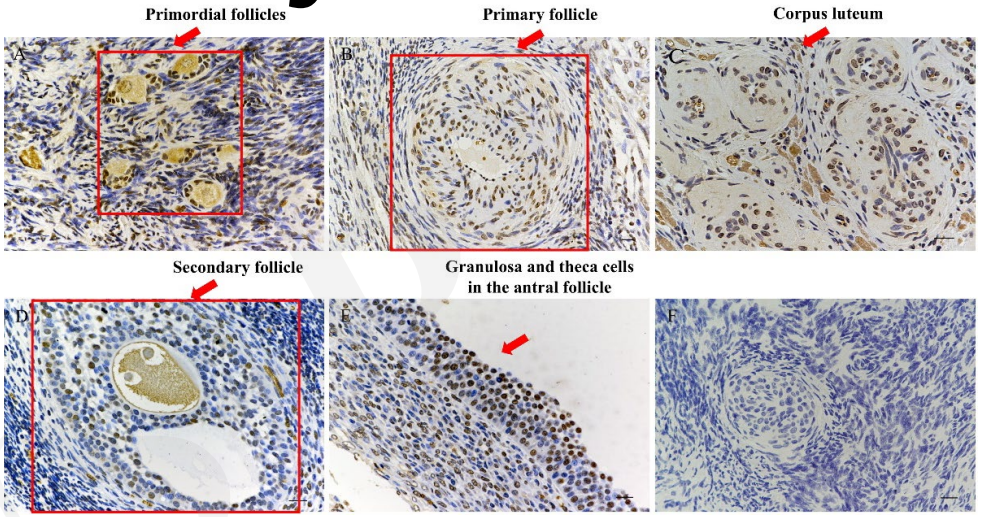

Figure 1

- **Western blotting**

We found greater CTSD protein expression in the ovary of monotocous goats than polytocous goats

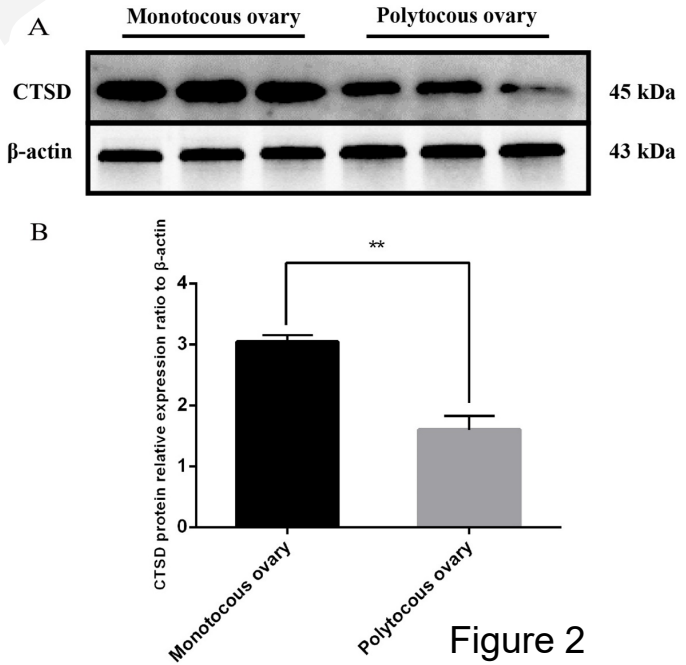

Figure 2

3 Research Summary

- Downregulation of CTSD expression in granulosa cells could significantly improve the expressions of BMPR-IB, FSHR and INHA at both transcriptional and translational levels

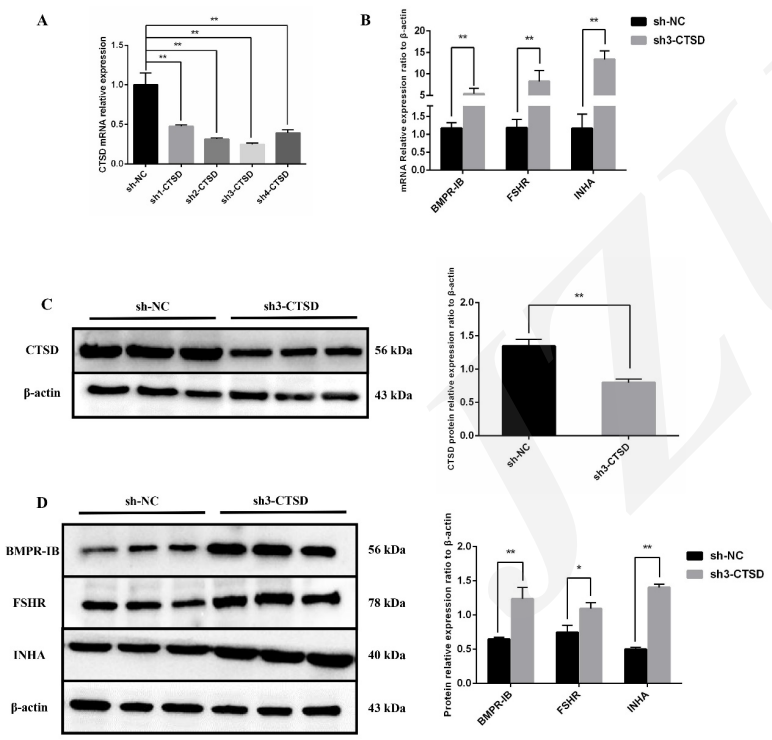

Figure 3

- CTSD knockdown significantly improved the proliferation of granulosa cells and increased the expression level of PCNA genes. sh3-CTSD demonstrated inhibition effect on cell apoptosis, and the levels of Bcl-2 were significantly higher in granulosa cells after CTSD knockdown. the expression of Bax and Caspase-3, were obviously reduced.

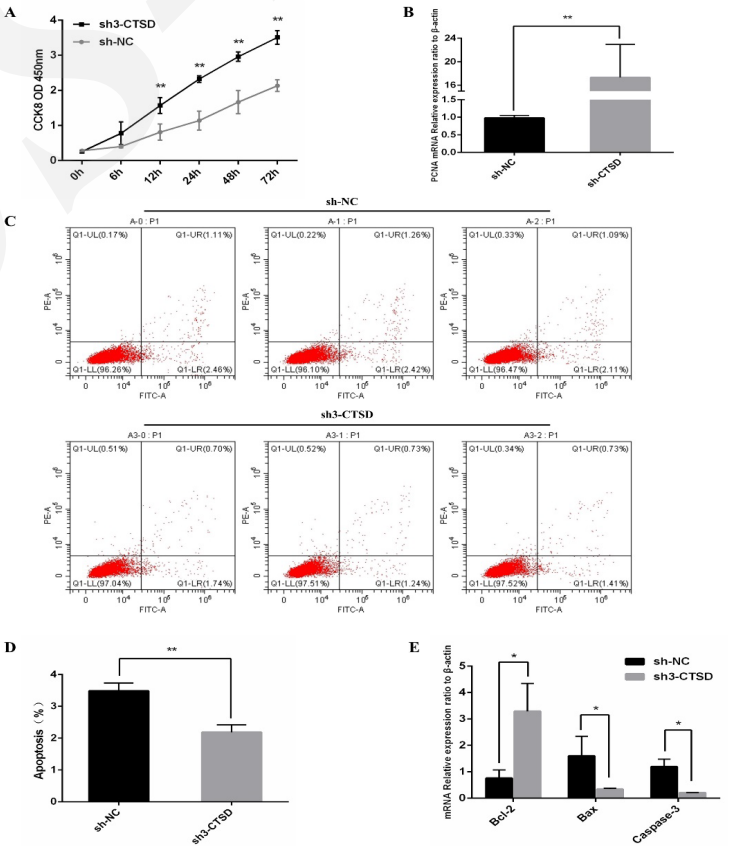

Figure 4

3 Research Summary

CTSD knockdown could significantly lower the ratio of granulosa cells in the G0/G1 and G2/M phases, whereas the ratio of cells in the S phase was obviously increased. In addition, CT SD knockdown demonstrated signifi cantly lower expression of the con- version factor Cyclin A1 from the S phase to the G2/M phase. In contrast, the expressions of a crucial cell cyc le protein, Cyclin D2, in the G1 phase and the key con version factor Cyclin E in cells from the G0/G1 to the S ph- ase were significantly and extremely high.

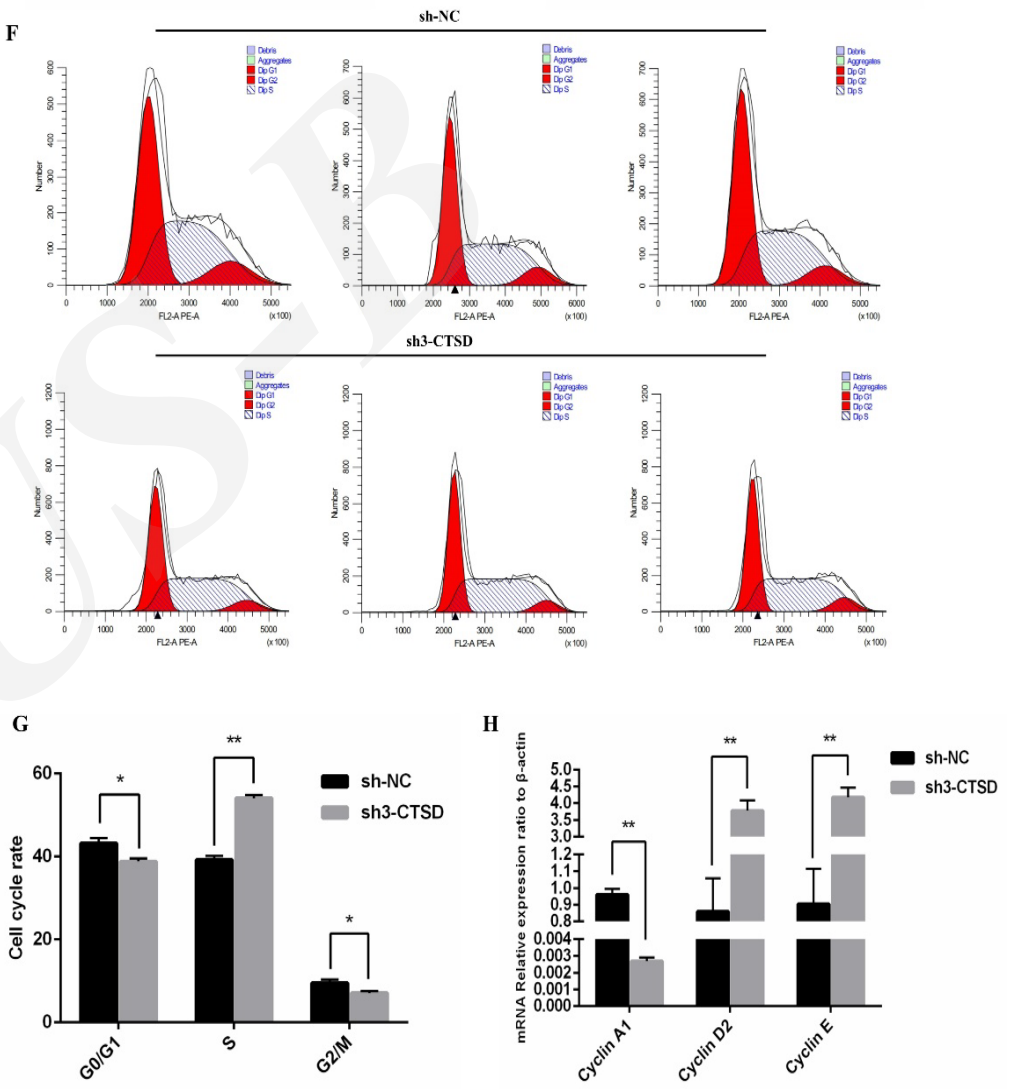

Figure 4