



Targeting *GNAQ* in hypothalamic nerve cells to regulate seasonal estrus in sheep

Mengting Zhu^{a, b, 1}, Hongmei Zhang^{c, 1}, Hua Yang^{a, *}, Zongsheng Zhao^{b, **}, Hugh T. Blair^d, Huihui Liang^b, Pei Wu^b, Qian Yu^a

^a State Key Laboratory of Sheep Genetic Improvement and Healthy Production, Xinjiang Academy of Agricultural and Reclamation Science, Shihezi, China

^b College of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang, China

^c First Affiliated Hospital, School of Medical College, Shihezi University, Shihezi, Xinjiang, 832008, China

^d Institute Veterinary, Animal & Biomedical Sciences, Massey University, Auckland, Palmerston North, New Zealand

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ABSTRACT

Kazakh sheep are typical seasonal estrus animals. Their reproductive system regulation mainly involves the complex regulation of the hypothalamic–pituitary–gonadal axis (HPGA), which is also closely related to reproductive hormone secretion. Gonadotropin-releasing hormone (GnRH), synthesized and secreted by the hypothalamus, is the key to controlling sheep reproductive activity. We studied how *GNAQ* (G protein subunit alpha q) regulates estrus in sheep by controlling GnRH expression and secretion. We used hypothalamic nerve cells as the research model. *GNAQ* overexpression and RNA interference vectors were constructed and transfected into the hypothalamic nerve cells of fetal Kazakh sheep. qPCR, western blotting, and enzyme-linked immunosorbent assay were used to detect *GNAQ* gene expression in Kazakh ewe tissues and analyze its regulatory effect on GnRH expression in the hypothalamic nerve cells. The fetal sheep hypothalamic nerve cells were successfully isolated and cultured. qPCR and cell immunofluorescence showed that the purity of positive cells was >95%. The tissue expression profile showed that there were different degrees of *GNAQ* gene expression in the Kazakh ewe tissue. Expression levels were relatively higher in the hypothalamus, pituitary, brain, and uterine tissues. When *GNAQ* expression was downregulated in the hypothalamic nerve cells, the upstream genes *KISS1* (kisspeptin), *GPR54* (KISS1 receptor), and *ER* (estrogen receptor) were all upregulated, as were the downstream genes *PLCB1* (phospholipase C beta 1), *PRKCB* (protein kinase C beta), and *GNRH*. At the same time, GnRH secretion levels were also upregulated. *GNAQ* regulated its downstream gene *PLCB1* in the hypothalamic nerve cells, and directly regulated GnRH expression and secretion through the calcium and PRKC signaling pathways. *GNAQ* also regulated kisspeptin expression, subsequently regulating GnRH expression and secretion indirectly through the kisspeptin–GPR54 signaling pathway. Our results are of great importance for improving the reproductive performance of seasonal-estrus sheep.

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1. Introduction

China is a major producer and consumer of mutton. The production of mutton in China has increased in recent years and is ranked first in the world. However, the shortage of mutton-based sheep products in China has increased the price of mutton. This

has increased local demand for mutton, and the discrepancy between high demand and low domestic production capacity is now prominent. It is, therefore, important to increase the reproductive performance of ewes to enhance mutton production.

Most Xinjiang local-breed sheep are seasonally bred (e.g., Kazakh sheep). The characteristics of seasonal estrus, ovulation, and lambing are the main reasons for the low efficiency of the entire Xinjiang sheep breeding industry. It also greatly affects the balanced supply of mutton throughout the seasons, which is a the main bottleneck of modern sheep breeding. Sheep seasonal estrus is mainly regulated by hormones secreted by the hypothalamic–pituitary–gonadal axis (HPGA) [1]. As the upstream organ of

* Corresponding author.

** Corresponding author.

E-mail addresses: yhxjcn@sina.com (H. Yang), zhaozongsh@shzu.edu.cn (Z. Zhao).

¹ These authors contributed equally to this work.