Expression of Cell Proliferation Regulatory Factors Bricd5, Tnfrsf21, Cdk1 Correlates With Expression of Clock Gene Cry1 in Testis of Hu Rams During Puberty

Yongjie Huang

Huazhong Agriculture University

Xun Ping Jiang

Huazhong Agriculture University

Guiqiong Liu (✉ liuguiqiong@mail.hzau.edu.cn)

Huazhong Agricultural University

Chen Hui Liu

Huazhong Agriculture University

Research Article

Keywords: cry1, bricd5, tnfrsf21, cdk1, tcfl5, Spermatogenesis

Posted Date: July 28th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-695426/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Expression of cell proliferation regulatory factors *bricd5*, *tnfrsf21*, *cdk1* correlates with expression of clock gene *cry1* in testis of Hu rams during puberty

Yongjie Huang^{1,2, #}. Xun Ping Jiang^{1,2, #}. Guiqiong Liu^{1,2, *}. Chen Hui Liu^{1,2}

¹ Laboratory of Small Ruminant Genetics, Breeding and Reproduction, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, People's Republic of China.

² Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of the Ministry of Education, Wuhan 430070, People's Republic of China.

Correspondence

[#]These authors contributed equally to this work.

^{*}Corresponding author.

^{*} Dr. Guiqiong Liu, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan 430070, People's Republic of China. Tel: +86-27-87585120; Fax: +86-27-87585120; E-mail: liuguiqiong@mail.hzau.edu.cn

Abstract—The *cry1* gene plays an important role in mammalian ontogeny and widely exists in various tissues. Studies showed that *cry1* gene was expressed in testis and participated in the regulation of mammalian reproductive activities. To identify the genes that are positively correlated with *cry1* gene expression in the testis of rams during the first estrus, and to explore the relationship between *cry1* gene and the number of spermatogenic cells. qRT-PCR was used to detect the mRNA transcription levels of *cry1*, *bricd5*, *tnfrsf21*, *cdk1* and *tcfl5* in testicular tissues of Hu sheep at 0, 30, 60, 90, 120, 150 and 180 days postpartum (dpp) . In testicular tissue, the expression of *cry1* mRNA increased with age in testis showed an upward trend, and increased significantly in the estrous phase. The *cry1* mRNA at 180dpp was significantly higher than that of 90-day-old testis ($p < 0.05$). The expression of cell proliferation related genes *bricd5*, *tnfrsf21*, *cdk1*, *cry1* upstream specific transcription factor *tcfl5* was identified, and the mRNA expression at 180 dpp was significantly higher than that of 3-month-old testis ($p < 0.05$) The expression level of *cry1* mRNA was similar to that of *cry1*. *cry1* gene is highly expressed in the testis of sheep during puberty, and has a significant correlation with the proliferation of spermatogenic cells.

Key words: *cry1*·*bricd5*·*tnfrsf21*·*cdk1*·*tcfl5*·Spermatogenesis

Introduction

The first estrus period of male animals refers to the first estrus of male animals and the release of fertilized sperm, which shows a complete behavioral sequence. It is a sign of animals from birth to reproductive capacity. The external performance of estrus is that the body develops most rapidly and produces sexual impulse; the internal performance is that the release of hypothalamic gonadotropin-releasing hormone and the secretion of anterior pituitary hormone begin to strengthen, and the production of androgen in testis increases, which promotes the production of mature germ cells [1]. The occurrence of estrus affects the life-long reproductive performance of livestock and poultry [2], in which spermatogenesis is an important part of the occurrence of estrus.

So far, 14 clock genes have been found, namely *clock*, *bmal1*, *per1*, *per2*, *per3*, *dec1*, *dec2*, *cry1*, *cry2*, *tim*, *ck1ε*, *rora*, *rev-erba* and *npas2*. The clock gene including *cry1* gene

is expressed in the suprachiasmatic nucleus of hypothalamus and almost all peripheral tissues of mammals [3]. Different clock genes can affect the physiological and biochemical functions of cells by regulating different downstream clock genes. In recent years, the research on the function of clock gene in mammalian reproductive organs such as ovary, uterus and testis has become a hot spot [4-7], indicating that clock gene is related to mammalian reproductive activities [8]. The change of clock gene expression in reproductive organs can affect the level of steroid reproductive hormones [9-10], and may affect sperm motility and spermatogenesis. In recent years, there have been similar reports that the Clock gene can affect the activity of acrosin activity [11]; CLOCK combines with SF3B3 and RANBP9 proteins to form a complex, and participates in the alternative splicing of genes related to spermatogenesis [12].

The *cry1* gene is one of the important negative feedback genes in the biological clock system. In addition to participating in the regulation of circadian rhythm, it may also be related to DNA damage repair, adipogenesis, follicular development and spermatogenesis. For example, the *cry1* gene regulates the classical Wnt/ β - catenin signaling pathway regulates adipogenic differentiation; after knockout of mouse *cry1* gene, the number of spermatogenic cell apoptosis increased and sperm count decreased significantly [13]; And, *cry1* gene, as a negative regulator of hypoxia inducible factor HIF1- α , participates in carcinogenesis [14].

It's found that *cry1* gene was mainly expressed in Leydig cells, Sertoli cells and spermatogenic cells in mouse testis [13]; The similar result is also observed in other species, such as camels [15]. These results suggest that *cry1* gene may play an important role in spermatogenesis. Therefore, in this study, we detected the expression levels of *cry1* upstream transcription factor and related genes before and after puberty, and analyzed their expression patterns, and observed the number of spermatogenic cells at all levels of testicular immune tissue in Hu sheep, and analyzed the relationship between them. In order to explore the effect of testicular core clock gene on the development of spermatogenic cells in male sheep, the expression level of *cry1*, a core clock gene, was studied. The results are of great significance to clarify the function of *cry1* gene in the

initiation of estrus.

Materials and methods

Sampling

For this study, 21 male Hu sheep with average ages of 0, 30, 60, 90, 120, 150 and 180 days old were randomly selected and purchased from the Zhiqinghe agriculture and animal husbandry Co., Ltd. (Yichang, Hubei, China). Each age group comprised three sheep (Unpublished). The sheep were raised in the same environmental conditions. The sheep were sedated after intramuscular injection by using 0.1ml/kg su mian xin also known as xylazine hydrochloride (Shengda, Changchun, Jilin, China), and the testicles were surgically collected and weighed using an electronic balance. One testis was fixed with 4% paraformaldehyde, and the other was frozen immediately in liquid nitrogen and then stored at -80°C .

HE staining of testicular tissue

The testis was washed with 0.9% saline, fixed with 4% paraformaldehyde for 48h at room temperature and embedded for further histologic analysis. The tissues were sliced into sections of 5 μm thick and stained with HE, and the morphology of the testis was observed under Eclipse-Ci™ microscope (NIKON, Chiyoda, Tokyo, Japan). The testicular tissue sections were observed and photographed under 400 times microscope. 50 round seminiferous tubules were randomly selected from each individual to count the cell layers and spermatogenic cells at all levels in the seminiferous tubules. The types of spermatogenic cells were determined according to the morphological characteristics of spermatogenic cells.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from testis tissues by the standard procedure. According to the steps of transgene's transcript One step gDNA removal and cDNA synthesis supermax reverse transcription kit, RNA was reverse transcribed into cDNA. A. Gene specific primers were directed to cry1(NM_001129735) s:5'-CTGCGTCTACATCCTCGACC-3', as:5'-TCGCAGATTGGCATCAAGGT-3';bricd5(XM_027961184) s:5'-ACCTTTGTGCCAAGACTCCC-3', as:5'-AGCAGACGGACACACAGAT

G-3'; tnfrsf21(XM_012100789.3) s:5'-GCAATGGCCACGGTATTGAC-3', as:5'-CCGTGTACCCGTTGGAGAAA); cdk1(NM_001142508.1) s:5'-TTCAGAGCTTTGGGCACTCC-3', as:5'-CGAGAGCAGATCCAAGCCAT-3'; tcf15 (XM_012188858.3)s:5'-GAGAGACACAACCGCATGGA-3', as:5'-ATGCTGTGGTCCATTGCAGA-3'. The primers for RT-PCR were designed with the Primer3plus software(<http://www.primer3plus.com/cgi-bin/dev/primer3plus.cgi>). The relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ methods using β -actin as the reference genes.

Sequence Characterization of TF binding sites of *cry1* in sheep

The promoter sequences prediction, a 5.00 kb genomic region upstream of the sheep *cry1* gene, was carried out using Jaspar online tools(<http://jaspar.genereg.net/>). The transcription factor binding sites on the 5' flank of *cry1* gene and their corresponding transcription factors were obtained[16]. All transcription factors were transformed into corresponding gene names and gene numbers by using Biomart online tools(<http://asia.ensembl.org/biomart/martview>). Tbtools was used to analyze the expression of these genes in different stages of postnatal development of Hu sheep[17]. Go enrichment analysis by Panther website(<http://geneontology.org/>). Cattle (*Bos taurus*) gene bank with high homology with sheep was selected as the template, and the results of enrichment retained the genes with FDR value less than 0.05 significant enrichment [19]. Ggplot2 package of R language is used to draw go enrichment analysis results for visualization.

Statistical analysis

The statistical significance of the results was analyzed through one-way ANOVA followed by Duncan's multiple comparison. Statistical significance was defined at the level of $p < 0.05$. Analyses were performed by applying SAS® OnDemand for Academics (SAS Institute, Inc., Cary, NC, USA). Results were presented as means $\pm$ standard deviation (SD).

Results

Histological analysis of testis during different postnatal stages

The results of HE staining of testicular tissue from 1 to 180 days postpartum (dpp)

were shown in Figure 1. From the first day to the 60th day, there were only spermatogonia and Sertoli cells in the seminiferous tubules. At the 90th day, spermatogonia began to proliferate in some seminiferous tubules, and a few primary spermatocytes appeared. At the 120th day, there were cavities in the seminiferous tubules, and the number of spermatocytes, early spermatocytes and a few spermatozoa were significantly higher than those at the 90th day. The number of layers of spermatogenic epithelial cells increased and the number of spermatogenic cells at all levels increased.

Fig. 1 Testicular development of Hu sheep. **A, B, C, D, E, F** Light micrographs of the testis in the Hu sheep at 1,30,60,90,120,150 and 180 days. HE staining, magnification 40×.Bar indicates 50μm.**H** The changes in the counts of spermatogenic cells in seminiferous tubules are shown

***Cry1* and proliferation regulatory-related mRNA expressions in the testicular tissues at different postnatal developmental stages**

We measured the expression of *cry1*, *bricd5*, *tnfrsf21* and *cdk1* by real time PCR for the testis tissues of rams at 1-180 dpp. It can be seen that the expression level of *cry1* gene in Hu sheep testis showed an upward trend from 0-180 dpp, and the relative

expression level of *cry1* gene in Hu sheep testis at 6 months old was significantly higher than that at 90 dpp ($p < 0.05$); the relative expression level of *bricd5* gene in Hu sheep testis showed an upward trend after 90 dpp, and the relative expression level of *bricd5* gene at 120-180 dpp was significantly higher than that at 90 dpp. The relative expression of *tnfrsf21* gene in the testis of Hu sheep aged 0-180 dpp showed an upward trend, and the relative expression of *tnfrsf21* gene in the testis of Hu sheep aged 180 dpp was significantly higher than that in the testis of Hu sheep aged 90 dpp ($p < 0.05$); the relative expression of *cdk1* gene in the testis of Hu sheep aged 90 dpp showed an upward trend, and the relative expression of *cdk1* gene in the testis of Hu sheep aged 150 dpp was significantly higher than that in the testis of Hu sheep aged 90 dpp. The expression level was significantly increased ($p < 0.05$). At each time point, the same letters indicated no significant difference, while different letters indicated significant difference ($p < 0.05$).

Fig. 2 Genes positively correlated with *cry1* expression measured by real time PCR. The relative expression of **A** *cry1* **B** *bricd5* **C** *tnfrsf21* and **D** *cdk1* in sheep testes at 1-180 days are shown. ANOVA was performed within each age of sheep and for each gene. Columns marked with different letters are significantly different

The expression levels of *cry1*, *bricd5*, *tnfrsf21* and *cdk1* mRNA were detected by real-time PCR. It can be seen that the expression level of *cry1* gene in male Hu sheep testis showed an upward trend from 0-180 days old, and the relative expression level of

cryl gene in Hu sheep testis at 6 months old was significantly higher than that at 3 months old ($p < 0.05$); after 90 dpp, the relative expression level of *bricd5* gene in Hu sheep testis showed an upward trend, and the relative expression level of *bricd5* gene at 120-180 dpp was significantly higher than that at 90 dpp. The relative expression of *tnfrsf21* gene in the testis of Hu sheep aged 0-180 dpp showed an upward trend, and the relative expression of *tnfrsf21* gene in the testis of Hu sheep aged 180 days was significantly higher than that in the testis of Hu sheep aged 90 dpp ($p < 0.05$); the relative expression of *cdk1* gene in the testis of Hu sheep aged 90 dpp showed an upward trend, and the relative expression of *cdk1* gene in the testis of Hu sheep aged 150 dpp was significantly higher than that in the testis of Hu sheep aged 90 dpp. The expression level was significantly increased ($p < 0.05$). At each time point, the same letters indicated no significant difference, while different letters indicated significant difference ($p < 0.05$).

Correlation and regression relationship between *cryl* mRNA expression and the counts of spermatogenic cells

The Pearson correlation coefficients of *cryl* gene transcription level with spermatogonia, primary spermatocytes, secondary spermatocytes, sperm cells and sperm number were 0.54-0.69 ($p < 0.05$, $p < 0.01$, Supplementary Table 1).

Table 1 Regression relationship between the *cryl* expression and the number of spermatogenic cells

Types of germ cells	Regression model	<i>P</i> value and R^2
spermatogonia	$spt = 2.1085cryl + 18.8472$	$P_{spt} = 0.0007$, $R_{spt}^2 = 0.4441$
primary spermatocytes	$pri = 3.3342cryl + 5.2313$	$P_{pri} = 0.001$, $R_{pri}^2 = 0.4232$
secondary spermatocytes	$sec = 1.6368cryl + 2.0793$	$P_{sec} = 0.0007$, $R_{sec}^2 = 0.4433$
spermatid	$spe = 2.5977cryl + 2.9245$	$P_{spe} = 0.0019$, $R_{spe}^2 = 0.3884$
sperm	$spm = 1.4511cryl + 1.5436$	$P_{spm} = 0.0094$, $R_{spm}^2 = 0.2920$

**spt*, *pri*, *sec*, *spe* and *spm* are short for the numbers of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatid, sperm.

There was a significant regression relationship between the expression level of *cryl*

gene and the number of germ cells in testis of Hu sheep from birth to 180 dpp ($p < 0.05$). In the process of spermatogenesis, the fitting degree of regression model between *cryl* gene expression level and spermatogonia number, primary spermatocyte number, secondary spermatocyte number, spermatocyte and sperm decreased in turn ($p < 0.05$, $R_{spt}^2 = 0.4441$, $R_{pri}^2 = 0.4232$, $R_{sec}^2 = 0.4433$) (Table 1).

Classification of transcription factors of the 5' upstream region expressed in testis

A total of 144 transcription factors were screened within 5000 bp upstream of the start codon of *cryl* gene (Fig. 3a). According to the transcriptome sequencing data, the transcription levels of 144 transcription factors in the testis of Hu sheep aged 1-180 days were changed (Fig. 3A). Among the 144 transcription factors, 25 transcription factors such as *tcfl5*, *klf4*, *thap1* and *foxg1* were significantly correlated with the relative expression level of *cryl* gene in testis ($p < 0.05$), while 23 transcription factors such as *arnt*, *barx1* and *sp3* were inversely correlated with the relative expression level of *cryl* gene ($p < 0.05$). The functional annotation of these 144 transcription factors showed that most of them were related to mesenchymal stem cell differentiation, hormone biosynthesis, cell population proliferation and reproductive system development. Among them, *klf5* and *klf4* were up-regulated in hormone biosynthesis, *nrf1* and *egr1* were down regulated; *hesx1*, *fosl1* and *tcfl5* were up-regulated in reproductive system development, *arnt*, *lhx4*, *lhx9*, *junb*, *sp3*, *hoxd13*, *sox17*, *dlx3*, *tfap2c* and *lhx8* were down regulated; *tcfl5*, *foxg1*, *tfap2a* were up-regulated in cell proliferation, and *egr1*, *sp1* and *fosl2* were down regulated.

Fig. 3 The changes of transcription level of 5000 bp transcription factor upstream of *cryl* gene promoter in 0-180-day old Hu sheep testis. The red lines represent the transcription factors that are positively correlated with *cryl* gene transcription level in the testis of Hu sheep aged 0-180 dpp. The blue lines represent the transcription factors that are negatively correlated with the transcription level of *cryl* gene in the testis of Hu sheep aged 0-180 dpp

Discussion

The expression of *cryl* gene in testis is closely related to mammalian reproduction. However, the relationship between the expression of *cryl* and the counts of spermatogenic cells has not been studied. This study provides evidence for the expression of *cryl* gene in testis, and helps to understand the local role of *cryl* gene in testicular tissue of sheep.

The CRY1 protein was mainly distributed in the basement membrane and interstitial cells of sheep testis. Also, on the epididymis, the luminal epithelial cells, peritubular myoid cells and luminal sperm showed positive expression [18]. The expression of CRY1 protein in testis was also detected in other species, such as mice and camels [13,15]. These results indicate that *cryl* gene may play an important role in testicular development. The sperm counts decreased significantly, and the number of degenerated and apoptotic spermatogenic cells increased significantly upon *cryl* knockout [13], indicating that *cryl* gene may be involved in the apoptosis of spermatogenic cells.

In this study, there were cavities in seminiferous tubules in testis of male

Hu sheep at 120 dpp. The number of spermatocytes, early spermatids and a small number of spermatozoa in the seminiferous tubules of ovine testis at 120 dpp was significantly higher than that at 90 dpp. The results showed that the testis of Hu sheep entered the stage of rapid development from 90 days of age, and Hu Sheep could enter the puberty from 120 days of age. The rapid development of spermatogenic cells in testis during the first estrus has also been observed in other animals. At the onset of puberty (90 days old), large cross-sectional seminiferous tubules, thick spermatogenic epithelial cells and obvious sperm were observed [19], which was consistent with our observation in Hu sheep. The results showed that there were differences in the estrus stage among different breeds.

In this study, *cryl* gene showed a unique time expression profile in the postnatal testis of sheep. The results showed that the expression of *cryl* gene was significantly different in the testis of sheep at different stages of postnatal development. The *cryl* mRNA level in sheep testis was increased from the initial stage of estrus. This is consistent with the time when spermatogenic cells of sheep begin to develop rapidly in the early stage of estrus, and have a significant correlation with the number of spermatogenic cells, which is similar to previous research results [13]. In addition, the expression trend of *bricd5* and *tnfrsf21* in the testis of sheep after delivery was similar to *cryl* gene and had correlation with each other. We speculate that it may be related to the proliferation of spermatogenic cells. In fact, the number of testicular germ cells depends on the number of supporting cells [20]. The supporting cells play a key role in spermatogenesis, such as MAPK, AMPK, and TGF in testicular support cells- β / Smad signaling pathway regulates the proliferation and meiosis of reproductive cells, and the proliferation of supporting cells. The function loss of *cryl* inhibited the (MAPK) -Erk signaling pathway, which affected the proliferation of osteoblasts [22]. After the interference of *cryl* gene expression in testis supporting cells, the expression of MT1 protein

also decreased obviously [23]. After overexpression of *cry1* in ovarian granulosa cells, MT1 expression was up regulated [15]. Melatonin can affect the proliferation of supporting cells through MT1 [24], while supporting cells can release nutrients to affect the development of spermatogenic cells [25]. Therefore, we predict that *cry1* gene in Sertoli cells may affect Sertoli cell proliferation and secretion of nutritional factors through MAPK signaling pathway, and affect the development of spermatogenic cells.

In addition, after the knockout of *cry1* and *cry2* genes in mice, the expression of *weel* gene increased and inhibited cell proliferation [26]. Our qPCR data showed that the expression of *weel* increased before and after puberty, but there was no significant change. However, the expression of *cdk1* gene downstream of *weel* gene related to G2/M phase of cell cycle increased significantly during puberty, there was a significant regression relationship between *cdk1* gene and *cry1* gene. This suggests that up regulation of *cry1* gene expression may promote cell proliferation by regulating spermatogenic cell cycle. We also analyzed the transcription level of transcription factors that may bind to *cry1* promoter region, and found that upstream of *cry1* gene binds transcription factors related to cell proliferation, hormone synthesis and reproduction, such as *tcfl5*. It further indicated that there was a close relationship between *cry1* gene and spermatogenic cell proliferation. In conclusion, these studies suggest that the clock gene *cry1* may regulate cell proliferation at multiple levels, such as supporting cytokine secretion, G2 / M transition and expression regulation of genes related to cell proliferation regulation.

Conclusion

In conclusion, there is a significant causal relationship between the transcription level of *cry1* gene in Hu sheep testis and the number of spermatogenic cells. It is speculated that *cry1* gene may regulate the proliferation of spermatogenic cells by regulating the expression of cell proliferation related genes such as *bricd5*, *tnfrsf21* and

cdk1. Future studies should focus on the role of *cry1* gene in sheep testes and Spermatogenic cell proliferation.

Acknowledgements

We are thankful to Dandan Du and all members of the Jiang laboratory who provided expertise that greatly assisted the research.

Authors' Contributions

Conceptualization, Xunping Jiang and Gui Qiong Liu; Methodology, Yongjie Huang and ChenHui Liu; Investigation, Yongjie Huang; Supervision, Xunping Jiang; Writing – Original Draft, Yongjie Huang and Xunping Jiang; Writing – Review & Editing, all authors.

Funding

Supported by China Agriculture Research System of MOF and MARA(CARS-38) . The funding bodies played no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interest.

Ethical approval The study was conducted according to the guidelines of The Scientific Ethic Committee of Huazhong Agricultural University (HZAUGO-2018-006, 1 March 2018).

Consent for publication The manuscript has been read and approved by all named authors.

References

1. Moulla F, El-Bouyahiaoui R, Nazih R, Abdelaziz N, Zerrouki N, Iguer-Ouada M (2018) Characterization of the onset of puberty in Tazegzawt lambs, an endangered Algerian sheep: Body weight, thoracic perimeter, testicular growth, and seminal parameters. *Veterinary World* 11(7):889-894. <https://10.14202/vetworld.2018.889-894>
2. Patterson J L, Beltranena E, Foxcroft G R (2010) The effect of gilt age at first estrus and breeding on third estrus on sow body weight changes and long-term reproductive performance. *Journal of Animal Science* 88(7):2500-13. <https://10.2527/jas.2008-1756>
3. Yang G, Chen L, Grant G R, Paschos G, Song W L, Musiek E S (2016) Timing of expression of the core clock gene *Bmal1* influences its effects on aging and survival. *Science Translational Medicine* 8(324):324ra16. <https://10.1126/scitranslmed.aad3305>
4. Li Z, Li Y, Ren Y, Li C (2020) High ambient temperature disrupted the circ

- adian rhythm of reproductive hormones and changed the testicular expression of steroidogenesis genes and clock genes in male mice. *Molecular and Cellular Endocrinology* 500(13):110639. <https://10.1016/j.mce.2019.110639>
5. Meyer V (2014) Searching for the Testicular Clock: Comparing daily patterns of clock gene expression and the duration of the seminiferous epithelium cycle in three hamster species.
6. Shimizu T, Hirai Y, Murayama C, Miyamoto A, Miyazaki H, Miyazaki K (2011) Circadian Clock genes *Per2* and clock regulate steroid production, cell proliferation, and luteinizing hormone receptor transcription in ovarian granulosa cells. *Biochemical & Biophysical Research Communications* 412(1):132-135. <https://doi.org/10.1016/j.bbrc.2011.07.058>
7. Aleksandar Z, Baburski, Silvana A, Andric (2019) Luteinizing hormone signaling is involved in synchronization of Leydig cell's clock and is crucial for rhythm robustness of testosterone production†. *Biology of Reproduction* 100(5):1406–1415. <https://10.1093/biolre/iox020>
8. Mereness A L, Murphy Z C, Forrestel A C, Susan B, Chemyong K, Richards J S (2016) Conditional Deletion of *Bmal1* in Ovarian Theca Cells Disrupts Ovulation in Female Mice. *Endocrinology* 157(2). 913-927. <https://10.1210/en.2015-1645>
9. Zhang J, Liu J, Zhu K, Hong Y, Sun Y, Zhao X, Du Y, Chen Z (2016) Effects of BMAL1–SIRT1-positive cycle on estrogen synthesis in human ovarian granulosa cells: an implicative role of BMAL1 in PCOS. *Endocrine* 53(2):574-584. <https://10.1007/s12020-016-0961-2>
10. Li R, Cheng S, Wang Z (2015) Circadian Clock Gene Plays a Key Role on Ovarian Cycle and Spontaneous Abortion. *Cellular Physiology and Biochemistry* 37(3):911-920. <https://10.1159/000430218>
11. Cheng S, Xin L, Wang Y, Zhou J, Wang Z (2016) The circadian Clock gene regulates acrosin activity of sperm through serine protease inhibitor A3K. *Experimental Biology & Medicine* 241(2):205-215. <https://10.1177/1535370215597199>
12. Yang J, Zhang Z, Zhang Y, Zheng X, Lu Y, Tao D (2017) CLOCK interacts with RANBP9 and is involved in alternative splicing in spermatogenesis. *Gene* 642:199-204. <https://10.1016/j.gene.2017.11.007>
13. Chong L, Xiao S, Jie H, Liao X, Gang L (2018) *Cry1* deficiency leads to testicular dysfunction and altered expression of genes involved in cell communication, chromatin reorganization, spermatogenesis, and immune response in mouse testis. *Molecular Reproduction & Development* 85(1):325-335. <https://10.1002/mrd.22968>
14. Dimova E Y, Jakupovic M, Kubaichuk K, Mennerich D, Chi T F, Tamanini F (2019) The circadian clock protein CRY1 is a negative regulator of HIF-1 α . *iScience* 13:284-304. <https://10.1016/j.isci.2019.02.027>
15. Zhao S Q, Gao Y, Zhang Y, Yang X P, Yang Z (2021) cAMP/PKA/CREB signaling pathway-mediated effects of melatonin receptor genes on clock gene

- expression in bactrian camel ovarian granulosa cells. *Domestic Animal Endocrinology* 76(4):106609. <https://10.1016/j.domaniend.2021.106609>
16. Anthony M, Oriol F, Arenillas D J, Chen C Y, D Grégoire, Jessica L, Shi W Q, Casper S, Tan G, Rebecca W H, Allen W Z, François P, Boris L, Albin S, Wyeth W W (2016) Jaspas 2016: a major expansion and update of the open-access database of transcription factor binding profiles. *Nucleic Acids Research*, 44(D1), D110-D115.
17. Chen C, Rui X, Hao C, He Y (2018) TBtools, a Toolkit for Biologists integrating various HTS-data handling tools with a user-friendly interface. <https://doi.org/10.1101/289660>
18. Wang S, Zhao Shu, Liu Cai, Zhang Cai, Han Le, Zhu Li, Wang Rui (2021) Expression and localization of cry1 gene in male sheep reproductive axis. *Chinese Journal of Animal Science* 57 (03):97-103.
19. Bo D, Jiang X, Liu G, Xu F, Girmay S (2020) Multipathway synergy promotes testicular transition from growth to spermatogenesis in early-puberty goats. *BMC Genomics* 21(1):372. <https://10.1186/s12864-020-6767-x>
20. Orth J M, Gunsalus G L, Lamperti A A (1988) Evidence from sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. *Endocrinology* (3):787. <https://10.1210/endo-122-3-787>
21. Ni F D, Hao S L, Yang W X (2019) Multiple signaling pathways in Sertoli cells: recent findings in spermatogenesis. *Cell Death & Disease* 10(8):541. <https://10.1038/s41419-019-1782-z>
22. Zhou L, He J, Sun S (2019) Cryptochrome 1 Regulates Osteoblast Differentiation via the AKT Kinase and Extracellular Signal-Regulated kinase Signaling pathways. *Cellular Reprogramming* 21(3). <https://10.1089/cell.2018.0054>
23. Chen Jian (2019) Study on the gene function of yak cry1. Gansu Agricultural University.
24. Xu K, Wang J, Liu H, Zhao J, Lu W (2020) Melatonin Promotes the Proliferation of Chicken Sertoli Cells by Activating the ERK/Inhibin Alpha Subunit Signaling Pathway. *Molecules* 25(5):1230. <https://10.3390/molecules25051230>
25. Niu B, Bo L, Wu C, Jiang W, Hua J (2016) Melatonin promotes goat spermatogonia stem cells (SSCs) proliferation by stimulating glial cell line-derived neurotrophic factor (GDNF) production in sertoli cells. *Oncotarget* 7(47):77532-77542. <https://10.18632/oncotarget.12720>
26. Horst G, Muijtjens M, Kobayashi K, Takano R, Yasui A (1999) Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. *Nature* 398(6728):627-30. <https://10.1038/19323>

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryInformation.pdf](#)