Research article

Small RNA-seq and hormones in the testes of dwarf hamsters (*Cricetulus barabensis*) reveal the potential pathways in photoperiod regulated reproduction☆

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ABSTRACT

Photoperiod regulates the functions and development of gonadal organs of seasonally breeding animals, resulting in breeding peaks in specific seasons. miRNA plays an important role in the regulation of testicular physiological functions. However, the relationship between photoperiods and miRNA levels in testes has yet to be conclusively determined. We investigated testicular miRNA of striped dwarf hamster (*Cricetulus barabensis*) responses to different photoperiods (long daylength [LD], moderate daylength [MD], and short daylength [SD]) and the potential pathways involved in photoperiod regulated reproduction. Testicular weights and reproductive hormone levels were measured in each of photoperiod treatments after 30 days. The concentrations of testosterone (T) and dihydrogen testosterone (DHT) in testes and Gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) in serum were higher in MD than in the other two groups. Testicular weights were heaviest in MD. Small RNA-seq was performed for the testes of hamsters in three groups. A total of 769 miRNAs were identified, of which 83 were differentially expressed between LD, MD, and SD. GO and KEGG analysis of target genes revealed that some miRNAs influence testicular activities by regulating the pathways related to cell apoptosis and metabolism. Gene expression pattern analysis showed that the MAPK signaling pathway may be the core pathway for photoperiodic regulation of reproduction. These results suggest that moderate daylength is more suitable for hamster reproduction while long daylength and short daylength may regulate reproduction through different molecular pathways.

1. Introduction

To adapt to seasonal variations, temperate animals have evolved seasonal rhythms, including changes in development,

reproduction, and energy metabolism [1]. These life history characteristics, especially reproductive activities, are formed during long-term evolution and are often strictly regulated by photoperiod and the hypothalamic-pituitary-gonadal axis [2]. Since miRNAs are widely expressed in the testis and are key regulators of biological processes in animals [3], it is important to investigate photoperiod regulated reproduction and the mechanisms through which miRNAs are involved in testicular responses to photoperiod regulated reproduction.

Various factors, such as temperature, photoperiod, food, and natural enemies can affect the seasonal rhythms of mammals. For non-tropical mammals, photoperiod is more stable than other environmental factors [4]. Photoperiods can regulate melatonin secretion and changes in melatonin levels are associated with a series of changes in the hypothalamus, pituitary gland, thyroid gland and gonads, thus regulating animal growth and reproductive rhythms [5].

Rodents play an important role in maintaining ecosystem balance. However, various non-specific rodenticides often used to kill rodents in farmland and houses. This is extremely detrimental to ecosystems and public health [6,7]. The reason for this phenomenon is that people cannot accept the impact of rodent population fluctuation in farmland. Therefore, investigating the reproductive cycle in rodents and the molecular mechanisms involved will reduce the use of rodenticides, thus avoiding environmental pollution, bio-accumulation of toxic substances, and extinction of endangered rodent populations. The striped dwarf hamster (*Cricetulus barabensis*) is a small non-hibernating mammal that is distributed in the northern temperate zones of Asia. They live on farmland and grassland. They usually have two reproductive peaks; spring and autumn, while their reproduction is inhibited in winter. In hamsters, there are significant seasonal changes in gene expression in the hypothalamus, immune function regulation, and energy metabolism [8,9]. Therefore, hamsters are good models for studies on the effects of photoperiod in wild animals.

miRNA is a small single strand RNA produced by DNA transcription, with a length of 18–25 nucleotides. They are not translated into proteins, but play important roles in regulating other genes during protein synthesis [10]. miRNAs are found in various organisms. A study involving the Kazakh sheep showed that ovaries exhibit different miRNA expression profiles in different seasons [11]. Down-regulated miR-34a-5p enhances FGF21 signaling and promotes beige adipogenesis [12]. miR-1 can sustain muscle physiology by controlling V-ATPase complex assembly [13]. miR-9a modulates germline stem cell maintenance and ageing by regulating *N*-cadherin levels [14]. miRNAs can also be influenced by physiological states and diseases.

The purpose of our research is to reveal the rule of population fluctuation of striped dwarf hamsters in different seasons by clarifying the response of testicular miRNA to photoperiod, thus providing basis for the protection and control of rodent population. To investigate the effects of photoperiod on striped dwarf hamster reproduction, we compared the hormonal levels and testicular miRNA expression profiles in three different day-lengths. Differentially expressed miRNAs were identified and their target genes subjected to GO and KEGG pathway analyses. Correlations between miRNA levels and their target genes in reproductive and metabolic pathways were analyzed. Our findings elucidate on miRNA-mediated photoperiodic regulation of reproduction, behavior, immunity and metabolism in rodents.

2. Materials and methods

2.1. Ethical statement

All animal protocols were in accordance with Laboratory Animal Guidelines for the Ethical Review of Animal Welfare (GB/T 35892-2018) and were approved by the Biomedical Ethical Committee of Qufu Normal University (No. 2022061). This study was performed in compliance with all ARRIVE guidelines.

2.2. Animals and grouping

Striped hamsters were captured in Qufu in Shandong Province, China (35°47′4″N, 117°02′11″E). Water and food (standard mouse chow from Jinan Pengyue Experimental Animal Breeding Co., Ltd., China) were provided *ad libitum*. Hamsters were placed in a light controller with an adaptive treatment period of 12L:12D at $22 \pm 2\,^\circ$C and a relative humidity of $55 \pm 5\%$. Twenty four male hamsters weighing 29.1–35.5 g were randomized into three groups: LD (Long daylength group; 14L:10D; light from 04:00 to 18:00); MD (Moderate daylength group; 12L:12D; light from 06:00 to 18:00); and SD (Short daylength group; 10L:14D; light from 08:00 to 18:00). Light intensity was set at 150 ± 10 lx. After 30 days, all hamsters were killed by CO_2 asphyxiation. Blood and the right testes of the hamsters were collected at 22:00–23:00 for hormonal determination. The concentrations of testosterone (T) and dihydrogen testosterone (DHT) in testis homogenate and GnRH, FSH and LH in serum were measured by ELISA. Three testes were selected from each group for small RNA sequencing.

2.3. Small RNA sequencing and data analysis

Total RNA was extracted from testes using a Trizol reagent kit (Invitrogen, Carlsbad, CA, USA). The RNA molecules in a size range of 18–30 nt were enriched via polyacrylamide gel electrophoresis. After ligation with adapters and PCR amplification, a cDNA library was constructed from PCR products of 140–160 bp size and sequenced using Illumina HiSeq Xten from Gene Denovo Biotechnology Co. (Guangzhou, China).

To identify the miRNAs, all clean tags were aligned to miRNA precursors and mature miRNAs of *C. barabensis* and all animal species by search against the miRBase database successively (Release 22 http://www.mirbase.org/). To distinguish between the results obtained by the two steps, cgr- was added before the first step when naming miRNA. Using the mirdeep2 software, novel miRNA

candidates were identified according to their genomic positions and hairpin structures.

miRNA expressions were determined and normalized to transcripts per million (TPM). Differentially expressed miRNAs were identified by $p < 0.05$ and fold change>1.5.

Three software programs; Miranda (Version 3.3a), RNAhybrid (Version 2.1) and TargetScan (Version 7.0) were used for target predictions. Intersections of the results were selected as predicted miRNA target genes.

2.4. Functional analysis of miRNA and target genes

Gene expression patterns were analyzed to cluster genes with similar expression patterns for multiple samples. To evaluate the expression patterns, differentially expressed miRNAs from each group were normalized to 0, log2 (v1/v0), log2 (v2/v0), and clustered by STEM [15]. Clustered profiles with $p \leq 0.05$ were considered significant. Target genes for differentially expressed miRNAs and clustered profiles were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses.

2.5. qRT-PCR validation of sequencing data

Total RNAs were extracted from the testes using an RNAiso Plus kit (TaKaRa, Dalian, China). cDNA synthesis was performed using the miRNA 1st strand cDNA synthesis kit (AG, Hunan, China). qRT-PCR was performed using a SYBR Green Premix Pro Taq HS qPCR Kit II (AG, Hunan, China). The reference gene was U6 snRNA. Eight randomly selected miRNAs were examined using the $2^{-\Delta\Delta ct}$ method. The primers used for RT-qPCR (Sangon, Shanghai, China) are shown in Table 1.

2.6. Enzyme-linked immunosorbent assay (ELISA)

Weigh not less than 50 mg testicular tissue sample and add PBS (0.01 mol/l, pH = 7.2) at a ratio of 1:9, which is equivalent to adding 9 ml PBS to 1 g tissue. Homogenization was performed on ice with a tissue homogenizer. The supernatant was centrifuged at 5000 rpm for 15 min. Take the supernatant for inspection. The serum was stored at -80 °C and incubated at 37 °C for thawing prior to analysis. ELISA kits for GnRH (HY–30506K), FSH (cat. no.HY-40016K), LH (cat. no.B165021), testosterone (cat. no.B165583) and dihydrogen testosterone (cat. no.B165347) were obtained from HEGNYUAN Biotechnology Co., Ltd., Shanghai, China. The procedures were performed in strict accordance with the manufacturer's protocol.

2.7. Statistical analyses

SPSS version 26.0 was used for organ weight and hormonal statistical analyses. The data are presented as the mean ± SD. One-way analysis of variance (ANOVA) was used to determine the overall differences; Fisher's least significant difference (LSD) post hoc test was used to determine group differences. The differences were considered significant when $P < 0.05$.

3. Results

3.1. Comparisons of testicular weights and hormonal levels of striped dwarf hamsters subjected to different photoperiods

Testicular weights (Fig. 1a) and hormonal levels are the most direct indicators of testicular physiological states. Testosterone (Fig. 1b), dihydrogen testosterone (Fig. 1c), GnRH (Fig. 1d), FSH (Fig. 1e), and LH (Fig. 1f) of striped dwarf hamsters were evaluated. Testicular weights were low in LD and SD, compared to MD ($P < 0.05$). Concentration of T in MD was significantly higher than that in the LD and SD ($P < 0.001$, $P < 0.001$) and SD group was significantly higher than LD ($P < 0.01$). DHT in testes met the trend of MD > SD > LD ($P < 0.001$). These results showed that after 30 days of treatment, the photoperiodic effect has been reflected in the testes. The serous GnRH content of MD was significantly higher than LD ($P < 0.001$), but there was no significant difference between MD and SD. The content of FSH in serum was higher in MD than in SD and LD ($P < 0.05$, $P < 0.001$), however, there is no significant difference between SD and LD. The content of LH in serum was higher in MD than in SD and LD ($P < 0.001$, $P < 0.001$), and SD is significantly higher than LD ($P < 0.001$). These results suggested that photoperiod affects testicular physiology through the hypothalamic-pituitary-gonadal (HPG) axis.

Table 1

Primer sequences of miRNAs in *Cricetulus barabensis*.

name	sequence (5′ to 3′)	length (nt)	GC
U6	GCTTCACGAATTTGCGTGTC	20	50
cgr-miR-25-3p	CATTGCACTTGTCTCGGTCTG	21	52.4
cgr-miR-125a-5p	TCCCTGAGACCCTTTAACCTGT	22	50
cgr-miR-148b-3p	TCAGTGCATCACAGAACTTTGTAA	24	37.5
cgr-miR-196a-5p	TAGGTAGTTTCATGTTGTTGGGAA	24	36
miR-22-5p	AAGCTGCCAGTTGAAGAACTGT	22	45.5
miR-143-5p	TGAGATGAAGCACTGTAGCTCTTAA	25	40
miR-181-3p	ACATTCAACGCTGTCGGTGA	20	50
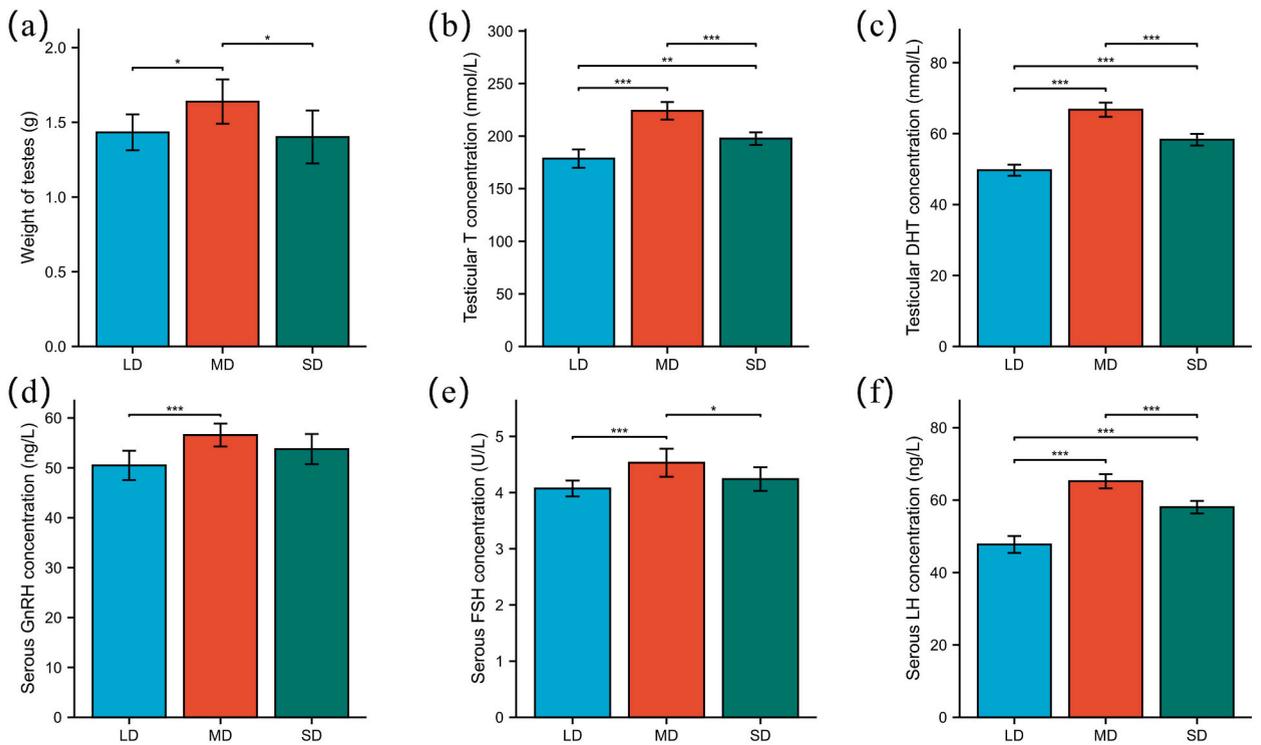
Fig. 1. Testicular weights and hormonal levels for *Cricetulus barabensis* under three photoperiod conditions. (a) Testicular weights under three photoperiod conditions (n = 8). (b) Testicular homogenate T levels in hamsters under three photoperiod conditions (n = 6). (c) Testicular homogenate DHT levels in hamsters under three photoperiod conditions. (n = 6) (d) Serous GnRH levels in hamsters under three photoperiod conditions. (n = 6) (e) Serous FSH levels in hamsters under three photoperiod conditions. (n = 6) (f) Serous LH levels in hamsters under three photoperiod conditions (n = 6). Values are expressed as means ± standard deviation; DHT dihydrogen testosterone; FSH, follicle-stimulating hormone; GnRH, Gonadotropin-releasing hormone; LD, long daylength; LH, luteinizing hormone; MD, moderate daylength; SD, short daylength; T, testosterone. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Fig. 2. Overview of small RNA sequencing and different expression profiles of miRNAs in testes under three photoperiod conditions. (a) Identification and length distribution of small RNA sequences. (b) Tags number of miRNAs with different lengths. (c) miRNA number in testes of *Cricetulus barabensis* under the three photoperiod conditions. (d) Differentially expressed miRNAs in each sample.

3.2. Overview of small RNA sequencing and different expression profiles of miRNAs

A total of 99665226 clean reads were obtained from the 9 miRNA libraries. After removing adapters and discarding sequences shorter than 18 nt, 97046365 clean tags were obtained. Then, 648 known miRNAs and 121 novel miRNAs were identified. The data of each sample are shown in Table s1 Sequence lengths of all known miRNAs and novel miRNAs as well as the tag number under this length were respectively counted (Fig. 2a and b). Most of the known miRNAs were concentrated in 21–24 nt, while novel miRNAs were distributed in 19, 22 and 25 nt.

Comparisons of all miRNA expressions revealed that 707 miRNAs were expressed in the three groups (Fig. 2c), 3 miRNAs were only expressed in SD and MD groups, and 7 miRNAs were only expressed in the LD group. There were 83 significantly differentially expressed miRNAs. Expression patterns for differentially expressed miRNA identified in *C. barabensis* are presented in a heatmap (Fig. 2d). Hairpin structures for differentially expressed novel miRNA sequences were predicted (Figure s1) and their sequences are shown in Table s2.

3.3. Target gene prediction and functional enrichment analyses

Taking the MD group as the reference, target genes for differentially expressed known miRNAs in LD and SD groups were predicted. In the LD group, 14205 targets were predicted by RNAhybrid, TargetScan and Miranda. In SD group, 7461 targets were predicted. GO analysis indicated that the enrichment of target genes of differential miRNA between LD and MD (Fig. 3a) was similar to that of SD and MD (Fig. 3b). In their enrichment maps, they have similar outlines in biological process, molecular function and cellular component. Only a few items are different, such as signaling and localization. KEGG analysis showed that the top 5 enriched pathways between LD and MD were; fluid shear stress and atherosclerosis, MAPK signaling pathway, longevity regulating pathway, TNF signaling pathway and glutathione metabolism (Fig. 4a). Between the SD and MD groups, metabolic pathways replaced the MAPK signaling pathway in

Fig. 3. GO enrichment analysis of targets. (a) GO enrichment of targets of the differentially expressed miRNAs between LD and MD groups. (b) GO enrichment of targets of differentially expressed miRNAs between SD and MD groups.

the top 5 (Fig. 4b). Although the top five pathways were relatively similar, however, there were great differences in the latter 15 pathways.

3.4. Gene expression pattern analysis

The STEM software was used for trend analysis and 8 expression trend figures were constructed (Fig. 5a). At the bottom of the picture are p-value, miRNA number and target number. Profiles 1, 2 and 6, which contained 29, 24 and 23 miRNAs were the three most prominent profiles. Trends for profiles 1 and 6 were exactly the same or opposite to those of testicular weight and hormonal levels. Only profile 1 shows the significance of gene enrichment ($p < 0.05$). Based on KEGG pathway information enriched by the target genes of each profile 1, the network diagram was drawn using interaction relationships between different pathways (Fig. 5b), which is conducive to finding the core pathway. The MAPK signaling pathway (ko04010) had more gene enrichments and high connectivity in profile 1, which may be the core pathway in the network.
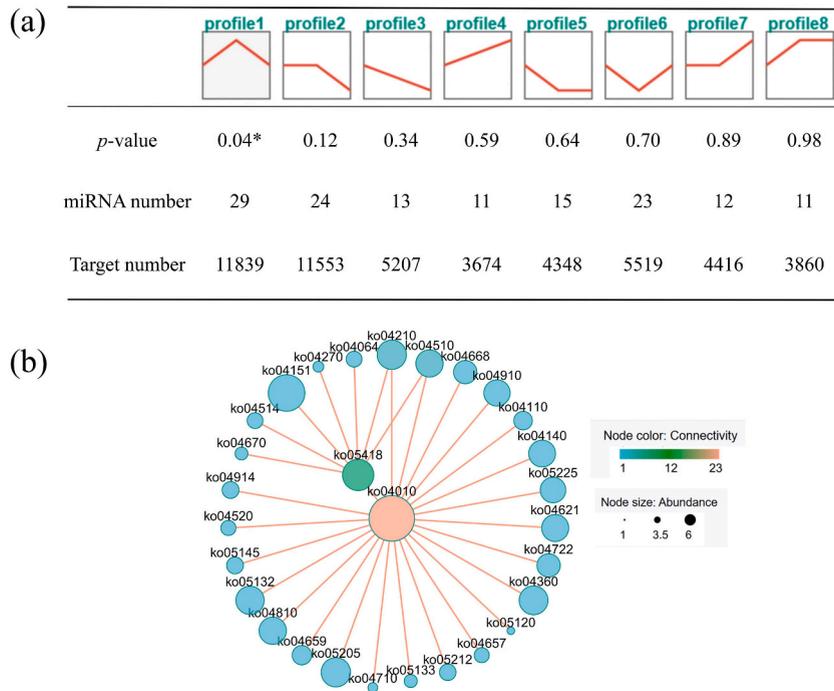
3.5. Validation of small RNA-seq data

To validate the small RNA-seq data, 8 miRNAs (cgr-miR-25-3p, cgr-miR-125a-5p, cgr-miR-148b-3p, cgr-miR-196a-5p, miR-22-5p, miR-143-5p, and miR-181-3p) were randomly selected for RT-qPCR analysis (Fig. 6). These miRNAs exhibited differences in expressions among the groups, validating the accuracy of small RNA-seq data.

4. Discussion

The striped dwarf hamster was assessed for The IUCN (International Union for Conservation of Nature) Red List of Threatened Species in 2016. Due to fluctuating hamster populations, there is a need to protect them and to prevent conflict with humans caused by population outbreaks in some areas. Therefore, it is of great significance to study the molecular mechanisms involved in the reproductive activities of striped dwarf hamsters and to formulate reasonable and effective protection measures. miRNA is one of the important factors in epigenetic regulation, and its role in post transcriptional regulation of genes has been documented. Thus, testicular
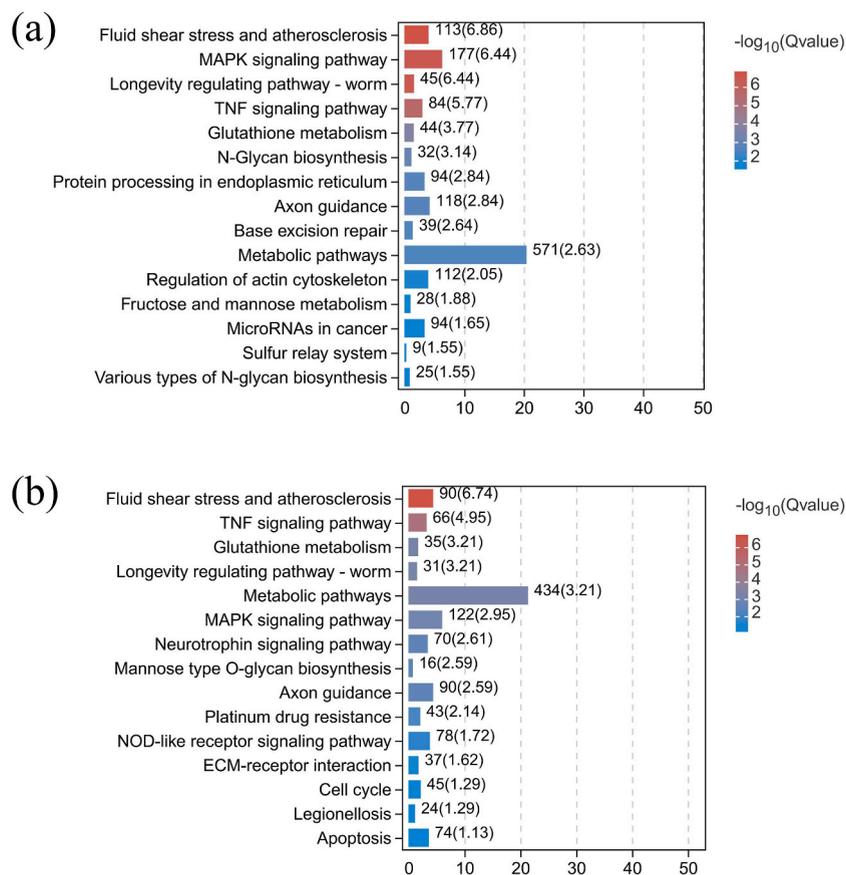
Fig. 4. **KEGG enrichment analysis of targets.** (a) KEGG enrichment of targets of differentially expressed miRNAs between LD and MD groups. (b) KEGG enrichment of targets of differentially expressed miRNAs between SD and MD groups.

Fig. 5. miRNA expression profiles and pathway relationships. (a) miRNA expression profiles. (b) Network of the interaction between pathways in profile 1.
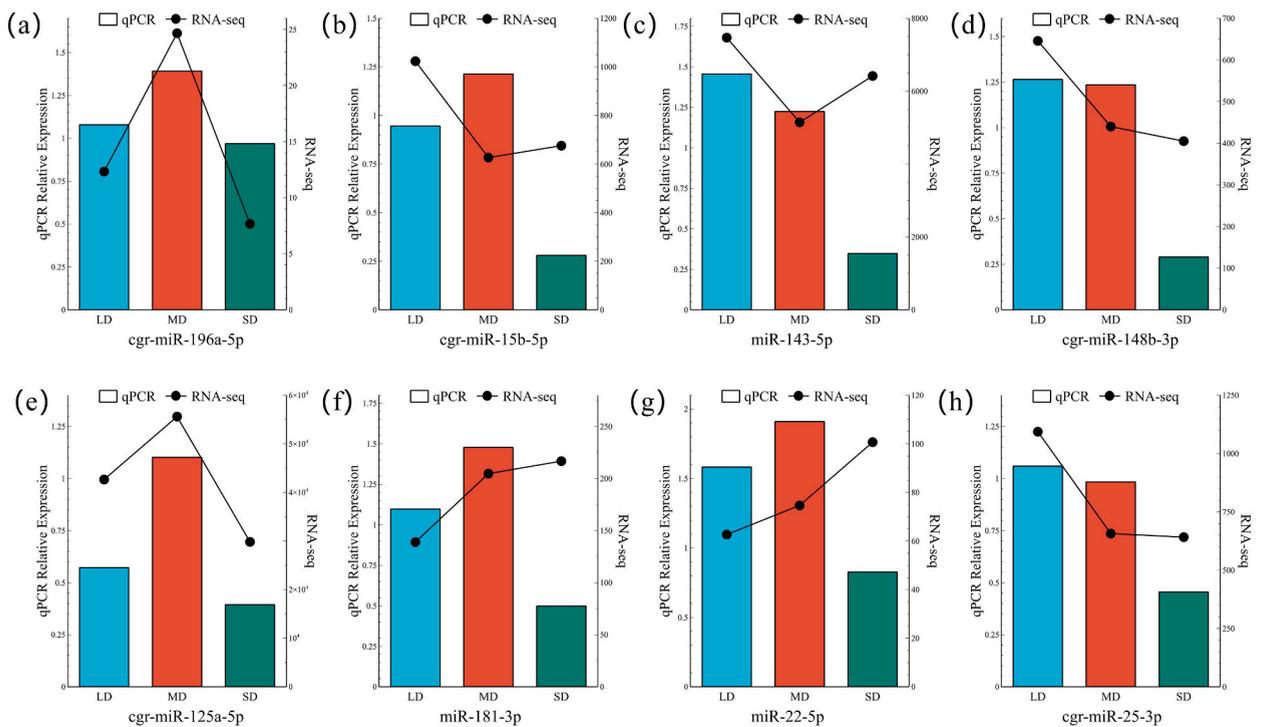
Fig. 6. qRT-PCR validation of the miRNA-seq data. (a) cgr-miR-196a-5p. (b) cgr-miR-15b-5p. (c) miR-143-5p. (d) cgr-miR-148b-3p. (e) cgr-miR-125a-5p. (f) miR-181-3p. (g) miR-22-5p. (h) cgr-miR-25-3p. Bar chart, qPCR relative expression; line chart, RNA-seq relative expression.

miRNA and reproduction related hormone levels of serum and testicular were measured in hamsters treated with different photoperiod.

The testes in the MD group were heavier. Reproductive states of animals are directly correlated with testicular weights. When the testes are large, animals are often in breeding period. Moreover, we found that testosterone and dihydrogen testosterone were highly concentrated in the MD group. Because they can maintain normal mating desire and reproductive function, it proves that hamsters in MD group were in breeding period. The content of GnRH, FSH and LH in serum suggest that photoperiod may affect testicular development and spermatogenesis through HPG axis. The upstream of HPG axis is melatonin and kiss1 neuron regulated by melatonin [16]. Therefore, a basic pathway between light and testis is formed. These findings are consistent with those of a previous study that reported that moderate daylength promotes breeding of striped hamsters. The medium length photoperiod would occur in spring and autumn, which is the right time for breeding to occur for small rodents with two breeding peaks However, the difference is that previous studies involving striped hamsters generally reported that short daylength inhibited breeding, while long daylength had no significant effects on breeding [17]. In this study, both long and short daylength showed breeding inhibition. The reason may be that the time scale for light treatment differs from that of other studies. Treatment for 6–8 weeks or more is often used [18]. This study confirmed that after 30 days of treatment, the testes under different photoperiods have indeed begun to show significant differences. Although this does not seem to achieve the maximum effect of photoperiod on testes, it is also of great significance to find and explore the initial stage of this effect.

The miRNA expression profiles in short, moderate and long photoperiods were obtained via a series of small RNA sequencing experiments to explore the causes of reproductive inhibition in hamsters under long and short daylength. A total of 648 known miRNAs and 121 novel miRNAs were identified. This is the first study on miRNA expression profiles in hamster testis in different photoperiods. Taking MD as the control, differences in known miRNA in the LD group were more than in SD, which was unexpected. In autumn and winter photoperiods, short-lived rodents suppress reproduction and molt to a thicker pelage. This is attributed to a significant increase of melatonin secretion from the pineal gland under short daylength [19]. Melatonin serves as a cue for changes in day length which brings about changes in the sensitivity to feedback mechanisms, thus helping hamsters to survive the harsh winter. A limited number of studies have evaluated the effects of long daylength on rodent reproduction. Small mammals such as rodents generally reproduce in the long days [20]. Although most studies believe long daylength promotes rodent reproduction, some researchers point out that long daylength can affect the spermatogenic cycle and inhibit spermatogenesis [21]. This divergence may be due to the differences in the duration and intensity of animal treatment. In order to maximize the photoperiod effect, animals were usually treated for more than 6 weeks. However, the disadvantage is that this will miss the initial stage of photoperiod effect and did not consider the phenomenon of photorefractory response [22]. It is understandable that different treatment duration leads to different results. The reproductive inhibition we observed may not be suitable for direct comparison with the results in other studies. Perhaps the reproductive inhibition caused by long daylength may have a faster recovery process, therefore, the testes of LD can return to the same state as MD after 6 weeks.

Among all the differentially expressed miRNAs, miR-10a-5p, miR-15b-5p and miR-125a-5p were highly abundant. miR-10a-5p is a steroid-responsive miRNA [23] that is highly expressed in semen [24]. At the same time, miR-10a is also expressed in fibroblasts [25]. This suggests that miR-10a has a role in interactions between germ cells and the microenvironment. We found that miR-10a-5p targets the *Btg1* gene, which has been reported to have an important role in cell proliferation [26]. This indicates that miR-10a-5p promotes cell proliferation, consistent with testicular weights and high expressions of miR-10a-5p in the MD group.

miR-15a can target *ccnt2* and play an important role in early spermatogenesis and meiosis [27]. miR-15b-5p is involved in NF- κB or mTOR signaling pathway or acss2/PTGS2 axis to regulate cell proliferation, autophagy and apoptosis [28].

miR-125a, a widely functional microRNA, is involved in physiological processes such as disease development, metabolism, and immunity among others [29]. miR-125a-5p is regulated by tissue inhibitors of metalloproteinase-1 (*TIMP-1*). *TIMP-1* knockdown increased miR-125a-5p expressions with a concomitant increase in apoptosis, and their interactions resulted in increased *p53* levels [30]. The relationship between miR-125a-5p and apoptosis explains testicular shrinkage of hamsters in short daylength. Moreover, miR-125a-5p inhibits glucose metabolism by targeting *STAT3* [31]. This may have an impact on the spermatogenesis process, which requires high energy levels.

According to the results of GO analysis, whether between LD and MD or SD and MD, the target genes of differentially expressed miRNAs are enriched in molecular functions, cellular components and biological processes, and the enrichment map is similar. Functional analysis of target genes revealed that miRNA is involved in regulation of the effects of photoperiods on physiological processes of hamster testicular cells. Comparable findings were obtained in buffalo and sheep [32,33].

KEGG analysis showed that the top 5 enriched pathways between LD and MD were; fluid shear stress and atherosclerosis, MAPK signaling pathway, longevity regulating pathway, TNF signaling pathway and glutathione metabolism. Between the SD and MD groups, metabolic pathways replaced the MAPK signaling pathway in the top 5. Unexpectedly, between SD and MD, there were three disease-related pathways (fluid shear stress and atherosclerosis, platinum drug resistance, legionellosis). This may be attributed to the different immune responses of hamsters [34]. Another possible explanation for this is that there may be a trade-off between immune functions and reproductive status, which has been supported by the winter immune enhancement hypothesis [35].

Gene expression pattern analysis is a method to cluster gene expression patterns according to the characteristics of multiple continuous samples. Then select the gene set that conforms to certain biological characteristics from the clustering results. So as to find the most representative gene group. This analysis divides the expression patterns of miRNAs that meet the threshold into 8 profiles. Among them, profiles 1, 3, 4, and 6, which are exactly the same or opposite to the physiological trend (testicular weight, hormone content) and light trend, may provide a reference for us to identify the key regulatory factors. Target genes for each profile were enriched in the KEGG pathway, and the network diagram was drawn through interactions between pathways. In profiles 0 and 5, the

MAPK signaling pathway was enriched and associated with other signaling pathways. The MAPK signaling pathway is involved in reproductive-related events such as sperm motility [36], gonadal development [37] and heat stress [38]. Therefore, the MAPK signaling pathway may be the core pathway for photoperiodic regulation of striped dwarf hamster testicular physiological states.

5. Conclusion

In this study, it was determined that striped hamsters secreted more reproductive related hormones under moderate daylength than long and short daylength in the time scale of 30 days. The miRNA expression profile of striped hamster testes in three daylength showed that the MAPK signaling pathway may be the core pathway for photoperiodic regulation of reproduction. Notwithstanding the relatively limited sample, this work offers valuable evidence of the effects of long daylength on the reproduction of striped dwarf hamsters.

Author contribution statement

Shuo Wang: Performed the experiments; Wrote the paper.
Jinhui Xu; Ming Wu: Analyzed and interpreted the data.
Xiangyu Zhao; Huiliang Xue: Contributed reagents, materials, analysis tools or data.
Yongzhen Feng; Wenlei Xu: Performed the experiments.
Laixiang Xu: Conceived and designed the experiments.

Data availability statement

Data associated with this study has been deposited at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (accession numbers: SRR18091424–SRR18091432).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e15687.

References

[1] T. Nakayama, T. Yoshimura, Seasonal rhythms: the role of thyrotropin and thyroid hormones, Thyroid 28 (1) (2018) 4–10, https://doi.org/10.1089/thy.2017.0186.

[2] Z. Wang, et al., Photoperiod affects harderian gland morphology and secretion in female *Cricetulus barabensis*: autophagy, apoptosis, and mitochondria, 408, Front. Physiol. 11 (2020) 408, https://doi.org/10.3389/fphys.2020.00408.

[3] L.F.R. Gebert, I.J. Macrae, Regulation of microRNA function in animals, Nat. Rev. Mol. Cell Biol. 20 (1) (2019) 21–37, https://doi.org/10.1038/s41580-018-0045-7.

[4] J.C. Borniger, R.J. Nelson, Photoperiodic regulation of behavior: Peromyscus as a model system, Semin. Cell Dev. Biol. 61 (2017) 82–91, https://doi.org/10.1016/j.semcdb.2016.06.015.

[5] K. Tsutsui, T. Ubuka, Photoperiodism in mammalian reproduction, in: M.K. Skinner (Ed.), Encyclopedia of Reproduction, second ed., Academic Press, Oxford, 2018, pp. 415–419, https://doi.org/10.1016/B978-0-12-809633-8.20586-4.

[6] J.A. Painter, et al., Salmonella-based rodenticides and public health, Emerg. Infect. Dis. 10 (6) (2004) 985–987, https://doi.org/10.3201/eid1006.030790.

[7] C. Frazzoli, Toxicological risk analysis in data-poor countries: a narrative approach to feed an "awareness raising-community empowerment" vortex, Medicina (Kaunas, Lithuania) 56 (11) (2020) 629, https://doi.org/10.3390/medicina56110629.

[8] L.X. Xu, et al., Seasonal differential expression of KiSS-1/GPR54 in the striped hamsters (*Cricetulus barabensis*) among different tissues, Integr. Zool. 12 (3) (2017) 260–268, https://doi.org/10.1111/1749-4877.12223.

[9] Z.J. Zhao, et al., Seasonal variations in metabolism and thermoregulation in the striped hamster (Cricetulus barabensis), J. Therm. Biol. 35 (1) (2010) 52–57, https://doi.org/10.1016/j.jtherbio.2009.10.008.

[10] S. Subramaniam, et al., Emergence of MicroRNAs as key players in cancer cell metabolism, Clin. Chem. 65 (9) (2019) 1090–1101, https://doi.org/10.1373/clinchem.2018.299651.

[11] M. Zhai, et al., Comparative profiling of differentially expressed microRNAs in estrous ovaries of Kazakh sheep in different seasons, Gene 664 (2018) 181–191, https://doi.org/10.1016/j.gene.2018.04.025.

[12] L. Rocha Andréa, et al., Enoxacin induces oxidative metabolism and mitigates obesity by regulating adipose tissue miRNA expression, Sci. Adv. 6 (49) (2020) eabc6250, https://doi.org/10.1126/sciadv.abc6250.

[13] P. Gutiérrez-Pérez, et al., miR-1 sustains muscle physiology by controlling V-ATPase complex assembly, Sci. Adv. 7 (42) (2021) eabh1434, https://doi.org/10.1126/sciadv.abh1434.

[14] Y. Epstein, et al., miR-9a modulates maintenance and ageing of *Drosophila* germline stem cells by limiting N-cadherin expression, Nat. Commun. 8 (1) (2017), https://doi.org/10.1038/s41467-017-00485-9.

[15] J. Ernst, Z. Bar-Joseph, STEM: a tool for the analysis of short time series gene expression data, BMC Bioinf. 7 (1) (2006) 191, https://doi.org/10.1186/1471-2105-7-191.

[16] A.S. Kauffman, Gonadal and nongonadal regulation of sex differences in hypothalamic Kiss1 neurones, J. Neuroendocrinol. 22 (7) (2010) 682–691, https://doi.org/10.1111/j.1365-2826.2010.02030.x.

[17] S.N. Li, et al., Photoperiod regulates the differential expression of KiSS-1 and GPR54 in various tissues and sexes of striped hamster, Genet. Mol. Res. 14 (4) (2015) 13894–13905, https://doi.org/10.4238/2015.October.29.10.

[18] J. Mou, et al., Effects of photoperiod on morphology and function in testis and epididymis of *Cricetulus barabensis*, J. Cell. Physiol. 236 (3) (2021) 2109–2125, https://doi.org/10.1002/jcp.29998.

[19] J.A. Evans, J.A. Elliott, M.R. Gorman, Individual differences in circadian waveform of Siberian hamsters under multiple lighting conditions, J. Biol. Rhythm. 27 (5) (2012) 410–419, https://doi.org/10.1177/0748730412455915.

[20] J.C. Walton, et al., Exogenous melatonin reproduces the effects of short day lengths on hippocampal function in male white-footed mice, *Peromyscus leucopus*, Neuroscience 248 (2013) 403–413, https://doi.org/10.1016/j.neuroscience.2013.06.020.

[21] V. Meyer, M. Klose, A. Lerchl, Spermatogenesis in the Roborovski hamster (*Phodopus roborovskii*) and the Chinese hamster (*Cricetulus griseus*), Andrology (2021), https://doi.org/10.1111/andr.13035 **n/a**(n/a).

[22] A. Herwig, et al., Hypothalamic ventricular ependymal thyroid hormone deiodinases are an important element of circannual timing in the Siberian hamster (*Phodopus sungorus*), PLoS One 8 (4) (2013), e62003, https://doi.org/10.1371/journal.pone.0062003.

[23] Z. Li, et al., miR-10a-5p, miR-99a-5p and miR-21-5p are steroid-responsive circulating microRNAs, Am. J. Tourism Res. 10 (5) (2018) 1490–1497.

[24] S. Fujimoto, et al., Distinct spectrum of microRNA expression in forensically relevant body fluids and probabilistic discriminant approach, 14332, Sci. Rep. 9 (1) (2019) 14332, https://doi.org/10.1038/s41598-019-50796-8.

[25] H. Yu, et al., Evolution of coding and non-coding genes in HOX clusters of a marsupial, BMC Genom. 13 (1) (2012) 251, https://doi.org/10.1186/1471-2164-13-251.

[26] J.P. Rouault, et al., BTG1, a member of a new family of antiproliferative genes, EMBO J. 11 (4) (1992) 1663–1670, https://doi.org/10.1002/j.1460-2075.1992.tb05213.x.

[27] Y. Teng, et al., Cyclin T2: a novel miR-15a target gene involved in early spermatogenesis, FEBS (Fed. Eur. Biochem. Soc.) Lett. 585 (15) (2011) 2493–2500, https://doi.org/10.1016/j.febslet.2011.06.031.

[28] Y. Zhu, et al., MALAT1/miR-15b-5p/MAPK1 mediates endothelial progenitor cells autophagy and affects coronary atherosclerotic heart disease via mTOR signaling pathway, Aging 11 (4) (2019) 1089–1109, https://doi.org/10.18632/aging.101766.

[29] Y. Qin, et al., MiR-125a Is a critical modulator for neutrophil development, e1007027, PLoS Genet. 13 (10) (2017), e1007027, https://doi.org/10.1371/journal.pgen.1007027.

[30] S. Ghoshal-Gupta, et al., TIMP-1 downregulation modulates miR-125a-5p expression and triggers the apoptotic pathway, Oncotarget 9 (10) (2018) 8941–8956, https://doi.org/10.18632/oncotarget.23832.

[31] L. Xu, et al., miR-125a-5p ameliorates hepatic glycolipid metabolism disorder in type 2 diabetes mellitus through targeting of STAT3, Theranostics 8 (20) (2018) 5593–5609, https://doi.org/10.7150/thno.27425.

[32] E. Capra, et al., Seasonal effects on miRNA and transcriptomic profile of oocytes and follicular cells in buffalo (*Bubalus bubalis*), Sci. Rep. 10 (1) (2020), https://doi.org/10.1038/s41598-020-70546-5.

[33] H. Yang, et al., Nutritional status affects the microRNA profile of the hypothalamus of female sheep, Reprod. Fertil. Dev. 30 (7) (2018) 946–957, https://doi.org/10.1071/RD17179.

[34] M.I. Parker, M.A. Palladino, MicroRNAs downregulated following immune activation of rat testis, Am. J. Reprod. Immunol. 77 (6) (2017), https://doi.org/10.1111/aji.12673.

[35] J.A. Sinclair, R. Lochmiller, The winter immunoenhancement hypothesis: associations among immunity, density, and survival in prairie vole (*Microtus ochrogaster*) populations, Can. J. Zool. 78 (2011) 254–264, https://doi.org/10.1139/z99-203.

[36] Z.Z. Yin, et al., Expression analysis of microRNAs and their target mRNAs of testes with high and low sperm motility in domestic pigeons (*Columba livia*), Genomics 113 (1) (2021) 257–264, https://doi.org/10.1016/j.ygeno.2020.12.024.

[37] X. Liu, et al., Identification and profiling of microRNAs during gonadal development in the giant freshwater prawn *Macrobrachium rosenbergii*, Sci. Rep. 9 (2019), https://doi.org/10.1038/s41598-019-38648-x.

[38] M. Rao, et al., Next-generation sequencing-based microRNA profiling of mice testis subjected to transient heat stress, Oncotarget 8 (67) (2017) 111672–111682, https://doi.org/10.18632/oncotarget.22900.