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Prolactin acts in the pathway of photoperiod regulating the reproduction of the Striped Hamsters

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Abstract

To study the effects of prolactin (PRL) in pituitary on the reproduction of the Striped Hamsters via the photoperiods. Mature female striped hamsters were raised under various photoperiods to study the differential expression pattern of PRL in pituitary, and prolactin receptor (PRLR) in the hypothalamus, pituitary and ovary. Photoperiods affect the pituitary PRL mRNA level and pituitary, hypothalamus and ovaries PRLR mRNA levels in striped hamsters. Positive correlations of PRL mRNA expression in the pituitary with that of PRLR in hypothalamus and ovaries, and that of GnRH in hypothalamus were determined. The serum concentration of PRL, FSH, LH and GnRH were significantly different among the striped hamsters from different photoperiods. Serum concentration of PRL is positively correlated with that of FSH, LH and GnRH of the striped hamsters. Photoperiod was important environment factor modulating the expression profile of PRL and PRLR in the striped hamsters.

Keywords: prolactin; photoperiod; reproduction; the striped hamsters;

Practical Application: The results may provide new ideas and methods for regulating the animal reproductive activities, and provide new targets to rationally control the population abundance of the rodents.

1 Introduction

It was first found that prolactin (PRL) was produced and secreted by horse in the glandular portion of the pituitary (Thompson & Oberhaus, 2015), and subsequently it was detected in the equine ovary by immunocytochemical techniques (King et al., 2010). The stimulatory effects of the extracts from bovine pituitary on rabbit mammary glands and a substance in similar extracts on pigeon crop sacs were respectively reported in 1928 (Stricker & Gruter, 1928) and Riddle et al. (1933) in 1933. For the function of the extracts which were related to milk inducing, it was termed as "prolactin". Subsequently, prolactin was also found to have the ability to promote breast development, maintaining lactation, and regulating animal reproduction (Zhang et al., 2012). In addition, prolactin was found in numbers of peripheral organs, such as heart, lung, spleen, liver, pancreas, kidney, adrenal gland, uterus, skeletal muscle, and skin (Bole-Feysot et al., 1998; Nagano & Kelly, 1994). Therefore, prolactin was considered as a unique hormone among pituitary hormones for its significant variation of the biological effects across species.

Prolactin receptor (PRLR) is a specific receptor for PRL. PRL binds PRLR and regulates the physiological processes including reproduction, development, metabolism, immunity, behavior and so on. Prolactin can bind PRLR in the hypothalamus to play its biological effects (Bole-Feysot et al., 1998; Clevenger et al., 2009). The expression of PRLR in the hypothalamus is increased by prolactin in serum (Sugiyama et al., 1994). In prolactin receptor knockout mice, luteal function is abnormal, and reproductive activities decrease with low ovulation rate, abnormal oogenesis, and implantation failure (Bole-Feysot et al., 1998). The binding of PRL with PRLR can regulate the synthesis of gonadotropin-releasing hormone (GnRH) in the hypothalamus, the secretion of lutein (HL), follicle stimulating hormone (FSH) and estrogen, and promote the maturation of cumulus cells, and further affect ovulation. The circulating levels of PRL are highly relevant to the levels of PRLR mRNA in both pituitary gland and hypothalamus, which indicates PRL positively regulates the PRLR expression in the hypothalamus (Leclerc et al., 2007).

However, the effects of prolactin on reproductive behaviors in females are different from various experiments. For example, some former studies show PRL suppresses the GnRH release in the highly differentiated GT1 GnRH cell lines (Milenkovic et al., 1994), however in vitro, endogenous hypothalamic PRL exerts a stimulatory effect on GnRH release (Azad et al., 1990). Endogenous increased prolactin caused by dopamine antagonism has no effect on mating behavior, but increased prolactin from nursing stimulus diminishes sexual behavior (Södersten et al., 1983). In addition, prolactin diminishes the sexual activity in the progesterone-primed ovariectomized rats injected estrogen from the third ventricle (Dudley et al., 1982), while prolactin enhances sexual activity in ovariectomized rats given estradiol from the midbrain (Harlan et al., 1983). The prolactin secretion has few or no effects on reproductive activity in the prairie vole (Smale et al., 1988). Therefore, the reproductive effect of prolactin is species and experiment designed-dependent.

Prolactin secretion is affected by a large variety of environment and the internal milieu. Photoperiod as the most reliable information to the time of year affects prolactin secretion by

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regulating the secretion of the pineal hormone melatonin (Curlewis, 1992). Photoperiod regulates the seasonal secretion of prolactin in mares (Johnson & Malinowski, 1986; Johnson, 1987) and Magang goose ganders (Shi et al., 2007). Prolactin is found to be with a season-dependent pattern for most seasonally breeding animals (Curlewis, 1992). But, some continuous breeders, such as the domesticated species, cattle and pigs, also show a seasonal change in plasma prolactin concentrations (Schams & Reinhardt, 1974; Ravault et al., 1982). So, the effect of PRL on the reproduction may be different between the seasonal and the yearly reproduction animals.

The striped hamster is one of main rodent pests in northern China farmland and has high reproductive capacity. It generally reproduces three times a year, and the litter size is of four to nine (Mu et al., 1999). The intensity of the reproductive activity is higher in spring (March to April) and autumn (September to October) than in summer (July to August) and winter (December to January) (Luo et al., 2000). The effect of prolactin on the seasonal reproduction of the striped hamster is not well known.

In this study, the female mature striped hamsters raised under various photoperiods (16L:8D, 12L:12D and 8L:16D light/dark cycle respectively) were used to study the differential expression pattern of PRL in pituitary, and PRLR in the hypothalamus, pituitary and ovary were tested by real-time quantitative PCR methods. The serum concentration of PRL, FSH and LH were also examined by enzyme-linked immunosorbent assay (ELISA) in Labsystems Multiskan MS 352. The correlations of the expression levels of PRL and PRLR mRNA with the serum concentration of PRL, FSH and LH were also analyzed. The results provide a theoretical basis for further understanding the seasonal reproduction mechanism of the striped hamster.

2 Materials and methods

2.1 Preparation for animals and tissues

The striped hamsters for this study were captured by iron cages as live-trap in the fields of Puwang village, which had been established to study the fluctuating mechanism of agricultural rodent populations for more than ten years. The village located in the northern plain of China which had typical warm temperate climates. The striped hamsters were captured in May of 2014. Firstly, the trapped striped hamsters were numbered uniquely; Secondly, they were gender identified, weighed, and appraised to provide information on size, age and reproductive condition; Thirdly, twenty-four mature female individuals were selected, whose body mass ranged from 22 to 24 grams, and were fed in the animal experiment center at Qufu Normal University with natural light irradiation and temperature conditions for one week. Fourthly, the selected twenty-four mature female individuals were randomly divided into three groups, and then raised under in long daylengths (16L:8 D, light: dark, LD), medium daylengths (12L:12D, light: dark, MD) and short daylengths (8L:16D, light: dark, SD) for two months respectively. Two months later, the estrous cycle of the striped hamsters was examined from 5 to 6 pm every day. When there was estrous behavior, the striped hamsters were immediately killed by CO_2 asphyxiation and dissected. The hypothalamus, pituitary, ovaries and testes were collected and stored at -80 °C. All experiments were approved by the Animal Ethics Committee of Qufu Normal University.

2.2 Total RNA extraction and RT-PCR

In this study, 8 mature female striped hamsters showing estrous behaviors were used from different photoperiods, and the deviation of the individual weights were all within 5% error of mean weight. Total RNA of the pituitary, the hypothalamus and the ovaries were extracted by using RNA extraction reagent (TaKaRa, Japan). The purity and the concentration of total RNA were assessed by the ratio of D260 to D280 using an ultraviolet spectrophotometer (Eppendorf, Germany). The integrity of total RNA was also examined by agarose gel electrophoresis (AGE). Subsequently, all the high-quality total RNA from the sampled tissues were reverse transcribed to cDNA using reverse transcriptase XL AMV with an Oligo deoxy thymidylate primer (TaKaRa, Japan). All synthesized cDNA was stored at -20 °C.

2.3 Real-time fluorescence quantitative PCR

Based on the nucleotide sequences (cloned by our research team, unpublished) of PRL,PRLR and GnRH for the striped hamsters, primers of real-time quantitative polymerase chain reaction (RT-qPCR) were designed using Beacon Designer 7.0 software. The primers for PRL were F1(5'- GACCGGGTGATCATGCTTTC-3') and R1 (5'-AGGAGTGCACCAAACTGAGT-3'), for PRLR were F2(5'- GGCCAACATGAAGGAAAGCA-3') and R2 (5'- TACCAGGATTCCACCAGCAG-3'), for GnRH were F3(5'-GAGCACTGGTCCTATGGGTT-3') and R3 (5'-ACATCTTCTTCTGCCTGGCT -3'). The primers for β-actin (as a reference gene) were F5 (5'-GAGACCTTCAACACCCCAGC-3') and R5(5'-ATGTCACGCACGATTTCCC-3'). RT-qPCR was performed using an Agilent Stratagene Mx3000P detector with the Brilliant II SYBR Green qPCR Master Mix (TaKaRa, Japan). Three repeats for each sample were performed during every RT-qPCR.

The volume of reaction system for RT-qPCR was 20 μL, including 10 μL SYBR Green, 0.4 μL forward primer and reverse primer (10 μmol/L) respectively, 2 μL cDNA template, 0.3 μL ROX and 6.9 μL DEPC H_2O. The reaction procedure was 10 min at 94 °C for enzyme activation followed by 40 cycles of 1 min at 94 °C, 1 min at 56 °C, and 1 min at 72 °C.

The fluorescence signal was collected during every PCR cycle at the renaturation step and was positively correlated with the quantity of the product. The melting curve analysis was used to confirm the specificity of the product with a single peak for each unique amplification, and the integrity of the product was examined by 2.5% AGE. The amplification efficiency for the specific primers was also tested using the standard curve, and it should be from 90% to 110% (Rutledge & Stewart, 2008). The expression level of PRLR mRNA was shown in the $2^{-\Delta\Delta C}{}_T$ way (normalization to β-actin) (Livak & Schmittgen, 2001).

2.4 Serum FSH and LH concentration determined

Blood samples were collected immediately after sacrifice and stored at 4 °C for about 30 min, after that centrifuged with 2400 g/min for 10 min at 4 °C. The serum concentration of PRL, GnRH, FSH and LH were measured by enzyme-linked immunosorbent assay (ELISA) in Labsystems Multiskan MS 352.

2.5 Statistical analysis

The means ± standard error were used to express effective data. The statistical analysis was conducted by using the independent sample t-test and the single-factor analysis of variance (ANOVA) of SPSS Statistics 17.0. The differences were considered to be significant when P< 0.05 and highly significant when P < 0.01. The Pearson's correlations of PRL, PRLR and GnRH relative expression levels with the serum concentration of PRL, GnRH, FSH and LH were analyzed by using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

3 Results

3.1 PRL and PRLR differential expression profiles of striped hamsters from different photoperiods

The expression variances of PRL and PRLR were demonstrated quantitatively in the striped hamsters from different photoperiods (Figure 1). Through $2^{-\Delta\Delta C}$T, results showed that the pituitary PRL mRNA level was higher in the striped hamsters from LD than that from MD and SD (P<0.01) (Figure 1a). The pituitary PRLR mRNA level was higher in the striped hamsters from LD than that from MD (P<0.05) and SD (P<0.01) (Figure 1b). The PRLR mRNA levels in the hypothalamus and ovaries were significantly different among LD, MD and SD (P<0.01) (Figure 1c and 1d), with the highest level in the striped hamsters from LD, and the lowest in individuals from SD.

3.2 Correlations the relative expression quantity of PRL in pituitary with that of PRLR and GnRH

The relationships of the relative expression quantity of RRL in pituitary with that of PRLR in pituitary, hypothalamus and ovaries, and that of GnRH in hypothalamus of the striped hamster were analyzed quantitatively (Figure 2). The significant positive correlations of PRL mRNA expression with that of PRLR in hypothalamus (r=0.780, P< 0.001) (Figure 2b) and ovaries (r=0.799, P< 0.001) (Figure 2c), and that of GnRH in hypothalamus (r=0.844, P< 0.001) (Figure 2d) of the striped hamster were determined, while no significant correlations were detected between the expression levels of PRL and that of PRLR in the pituitary (Figure 2a).

3.3 Serum concentration of PRL, FSH, LH and GnRH in the striped hamsters from different photoperiods

The serum concentration of PRL, FSH, LH and GnRH in the striped hamsters from different photoperiods were examined (Figure 3). Significant variations of the serum concentration of PRL, FSH, LH and GnRH existed in the striped hamsters from different photoperiods. The highest serum concentration of PRL, FSH, LH and GnRH were examined in the individuals

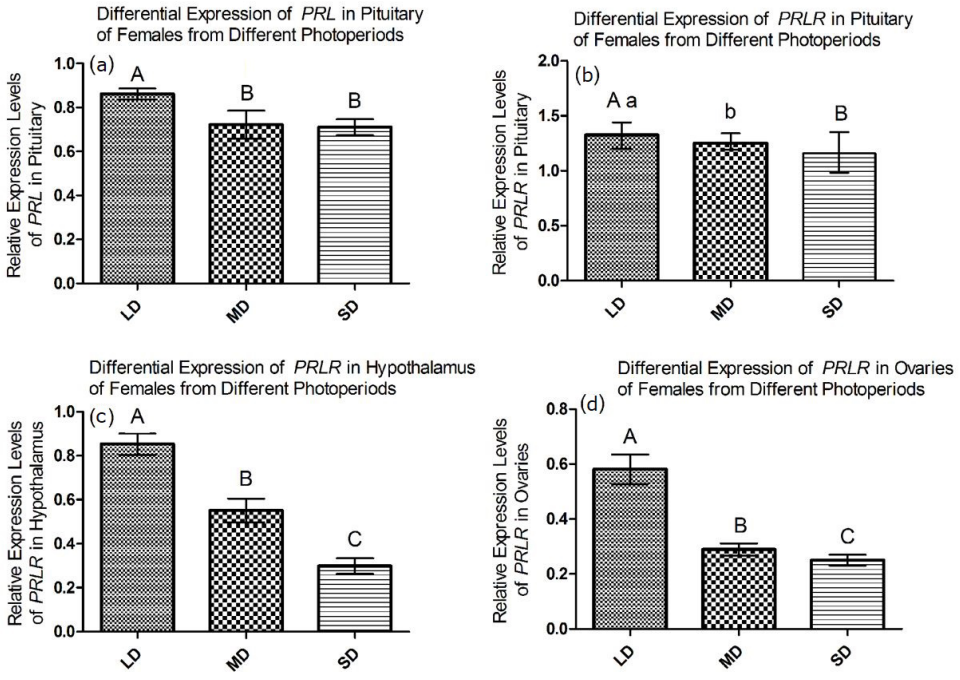

Figure 1. The relative expression levels of PRL and PRLR in the striped hamsters from different photoperiods. (a) The differential expression of PRL in pituitary in the striped hamsters from different photoperiods; (b) The differential expression of PRLR in pituitary in the striped hamsters from different photoperiods; (c) The differential expression of PRLR in hypothalamus in the striped hamsters from different photoperiods; (d) The differential expression of PRLR in ovaries in the striped hamsters from different photoperiods.

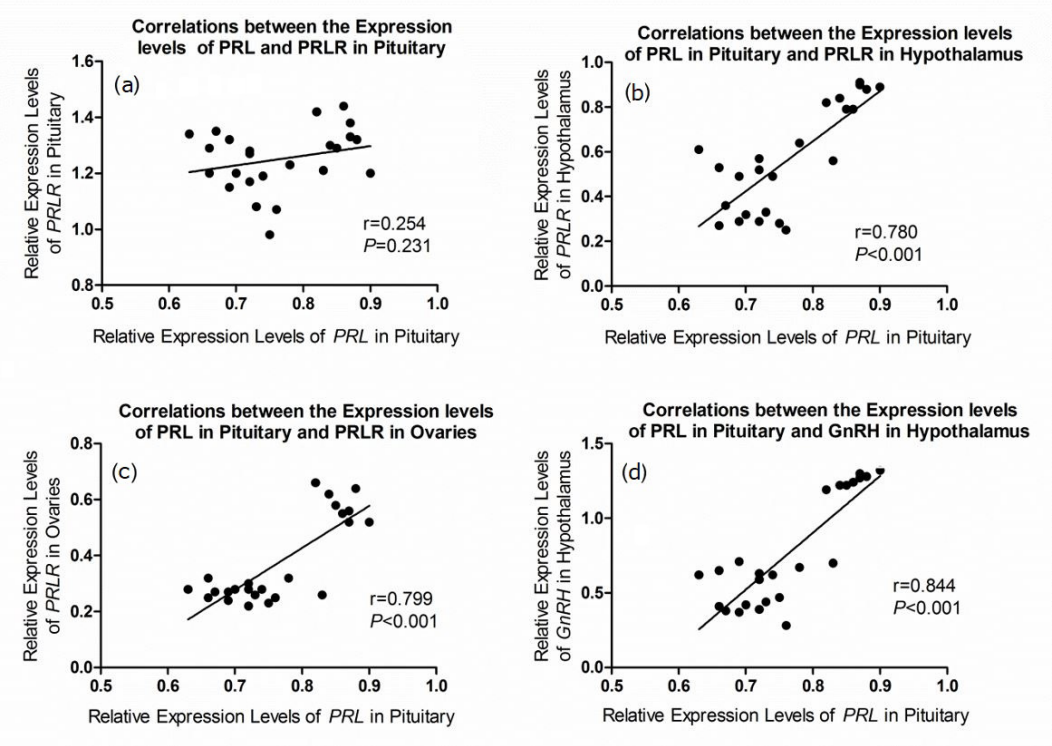

Figure 2. Relationships of the relative expression quantity of RRL in pituitary with that of PRL in pituitary, hypothalamus and ovaries, and that of GnRH in hypothalamus of the striped hamster. (a) Correlations of the expression levels between PRL and PRLR in the pituitary; (b) Correlations of the expression levels of PRL in the pituitary with that of PRLR in the hypothalamus; (c) Correlations of the expression levels of PRL in the pituitary with that of PRLR in the ovaries; (d) Correlations of the expression levels of PRL in the pituitary with that of GnRH in the hypothalamus.

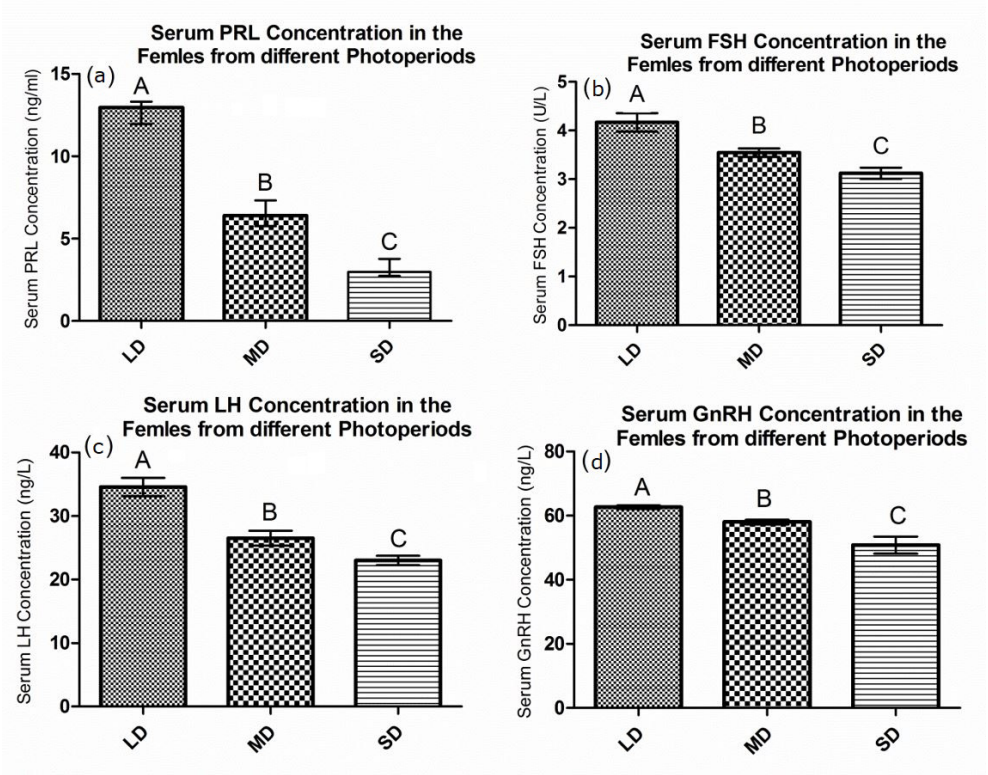

Figure 3. Different serum concentration of PRL, FSH, LH and GnRH in the striped hamsters from different photoperiods. (a) Different serum PRL concentration from LD, MD and SD conditions; (b) Different serum FSH concentration from LD, MD and SD conditions; (c) Different serum LH concentration from LD, MD and SD conditions; (d) Different serum GnRH concentration from LD, MD and SD conditions. Note: Different capital letters above columns show highly significant differences (at $P < 0.01$ level).

from LD (Figure 3), and the lowest in the striped hamsters from SD (Figure 3).

3.4 Relationships between the serum concentration of PRL to that of FSH, LH and GnRH

Correlations of the serum concentration of PRL to that of FSH, LH and GnRH of the striped hamster were detected (Figure 4). The serum concentration of PRL was positively correlated with that of FSH(r=0.949, $P< 0.001$) (Figure 4a), LH(r=0.965, $P< 0.001$) (Figure 4b) and GnRH (r=0.889, $P< 0.001$) (Figure 4c) of the striped hamster.

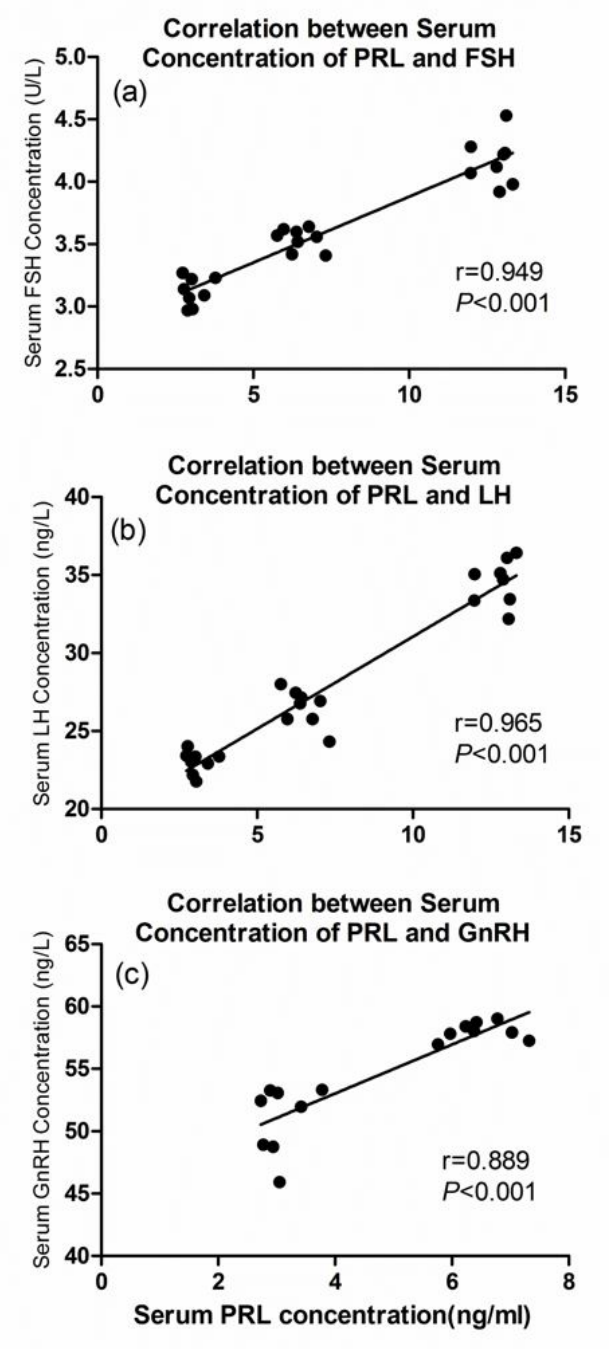

Figure 4. Relationships of the serum concentration of PRL to that of FSH, LH and GnRH of the striped hamster. (a) Correlations of the serum concentration of PRL with that of FSH; (b) Correlations of the serum concentration of PRL with that of LH; (c) Correlations of the serum concentration of PRL with that of GnRH.

4 Discussion

This study mainly demonstrated the expression profile of PRL in the pituitary of the striped hamsters under different photoperiods, which was in accordance with the previously reported expression profile of PRL in horses (Thompson & Oberhaus, 2015) and Magang goose ganders (Shi et al., 2007). Photoperiod is the major environmental factor by regulating the secretion of the pineal hormone melatonin to control the secretion of prolactin, increasing in long days but opposite effect in short days (Shi et al., 2007; Thompson & Oberhaus, 2015). For adult male hamsters, exposing to a short photoperiod (5 h of light, 19 h of darkness) for 2 months causes a significant decline in prolactin secretion (Badura & Goldman, 1997; Bex et al., 1978). Light is an important regulator of prolactin secretion in photoperiodic mammals, and the prolactin secretion rhythm is in coincident with the shift of the light (Blake, 1976). In the striped hamsters, the expression level of PRL in LD is significantly higher than those in SD conditions; our result is in consistent with the former studies photoperiod affecting the expression of PRLR.

Further more, this study also demonstrated that the expression profile of PRLR in the hypothalamus, pituitary and ovaries of the striped hamsters are also regulated by the photoperiods, and PRLR acts an important role in the reproduction of the striped hamsters, which is in consistent with the results of Bole-Feysot's, luteal function is abnormal and reproductive activity is missed for decreased ovulation rate, aberrant oogenesis, and implantation failure in prolactin receptor knockout mice (Bole-Feysot et al., 1998).

The expression level of PRL in pituitary could increase the expression of PRLR in the hypothalamus is also reported by the former study (Sugiyama et al., 1994; Leclerc et al., 2007). In this study, we found the serum concentration of PRL was highly correlated with levels of the PRLR mRNA in both the pituitary gland and hypothalamus of the striped hamsters, which indicated PRL positively regulated the PRLR expression in the pituitary and hypothalamus (Leclerc et al., 2007), and PRLR took part in the biological actions of PRL (Bole-Feysot et al., 1998), and PRL and PRLR regulated the reproductive activity of the striped hamsters.

The serum concentration of PRL or the expression levels of PRL in the pituitary are positively correlated with the serum concentration of GnRH, FSH and LH. The expression of PRLR in the hypothalamus is increased by prolactin in serum (Sugiyama et al., 1994).

In this study, PRL increased the expression level of GnRH in hypothalamus and serum concentration of GnRH in the striped hamster, which is in consistent with that endogenous hypothalamic PRL exert a stimulatory effect on GnRH release (Azad et al., 1990), and discrepancy with that PRL suppresses the GnRH release in the highly differentiated GT1 GnRH cell lines (Milenkovic et al., 1994). Some studies also show that the effect of PRL on plasma gonadotropin levels is sex (Calogero et al.,

1993) or dose-dependent (Milenkovic et al., 1994). So, PRL regulates the expression level of GnRH in complicated ways, and the effect of PRL on GnRH also may be related with species character or the various experiment designs.

In addition, this study domonstrated the positive correlation of PRL expression level in the pituitary with the serum concentration of FSH and LH, which is associated with the effect of PRL on animal reproduction (Zhang et al., 2012), while in the prairie vole, photoperiod markedly affects the prolactin secretion but has little or no effects on reproductive activity (Smale et al., 1988). The effect of PRL on the reproduction may be species -dependent.

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