Male reproductive cycle of hibernating Korean greater horseshoe bat, *Rhinolophus ferrumequinum korai* (Chiroptera: Rhinolophidae): annual cycle of the seminiferous epithelium and morphological changes of the testes

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**Abstract**

Morphological changes of testes and the annual cycle of the seminiferous epithelium were observed by optical and transmission electron microscopy to determine the male reproductive cycle of the Korean greater horseshoe bat, *Rhinolophus ferrumequinum korai*. In this study, *R. ferrumequinum korai* showed a distinct reproductive cycle, unlike other hibernating bat species. The testicular reproductive cycle of *R. ferrumequinum korai* consists of three main stages. The first is the spermatogenesis stage (from April to September), including spermatocytogenesis (which appears from April to May) and spermiogenesis (from June to September). The activity of spermatogenesis was highest in August. The lumen of seminiferous tubules was open from mid-April to mid-October. It was closed from November to March of the following year. The second stage is the phagocytosis stage (from mid-October to mid-November), which is a purification process to prepare for new spermatogenesis the following year. The third is the dormant stage – that is, a state of holding only spermatogonia and Sertoli cells. It is an adaptation strategy to store energy for long periods of hibernation. Compared to previous studies, spermatogenesis period preceded 1 month earlier. This suggests that temperature increase can impact reproductive development and spermatogenesis.

**Keywords:** Annual cycle of the seminiferous epithelium, cleansing period, phagocytosis

**Introduction**

Mammalian species have various breeding methods. Animals in temperate zones are known to limit their reproductive activities to specific periods in order to maximize progeny survival (Nakao et al. 2008). In particular, changes in daylight duration are main factors that affect the start of the breeding season. Many other factors such as estrogen levels, neuropeptides, kisspeptin, gonadotropin-releasing hormone/luteinizing hormone (GnRH/LH), thyroid, food, water, housing, space and climate availability also affect the onset of reproduction (Vasantha 2016).

The breeding period of hibernating bats is interrupted by hibernation. It only occurs at specific times. Breeding types of hibernating bats differ greatly from those of mammals in general. Hibernation is a physiological adaptation for long-term survival (Wimsatt 1960, 1969; Krutzsch 1979; Lee et al. 1993). It has a significant impact on fertilization (Oh et al. 1985a). Characteristics of fertilization and early embryogenesis are different among species. Since breeding is done under low-temperature environmental conditions, adaptation strategies such as efficient energy use during breeding are needed (Funakoshi & Uchida 1978).

A comprehensive description of male reproduction patterns in hibernating bats is needed because their reproduction events and patterns during hibernation are significantly different from those of other mammals. Their male reproductive cycles can be divided into different patterns based on the timing and duration of major events during the annual reproductive cycle (Courrier 1927; Wimsatt 1960, 1969). Male
reproductive patterns of bats have been classified into three types (“Pipistrellus pattern”, “Myotis pattern” and “Miniopterus pattern”) considering the spermatogenesis process, Leydig cell and associated organ changes (Gustafson 1979). In general, bats that live in temperate and tropical regions show a seasonal breeding pattern, although some species may not show such a pattern (Wilson 1979; Heideman & Bronson 1994; Crichton & Krutzsch 2000).

Recently, several studies have reported the periodicity of the seminiferous epithelium in bats, including spermatogenesis (Merwe & Rautenbach 1987; Morigaki et al. 2001; Kurohmaru et al. 2001; Kang & Lee 2004; Lee & Mōri 2004; Sharifi et al. 2004; Beguelini et al. 2009, 2011b, 2012a,b, 2013, 2014; Morais et al. 2012, 2013, 2014). Major events are similar in most species of bats. However, variations in the process between families and other taxa are often observed (Fawcett & Ito 1965; Singwi & Lall 1983; Breed & Leigh 1985; Oh et al. 1985b; Lee et al. 1992; Saidapur & Patil 1992; Phillips et al. 1997; Son et al. 1997; Kim et al. 1999; Beguelini et al. 2011a,b). In particular, there has been no report of extinguishment of immature spermatids due to phagocytosis of Sertoli cells during the annual cycle of the seminiferous epithelium except for the male reproductive type of R. ferrumequinum korai (Lee et al. 1993). Therefore, the objective of this study was to investigate the relationship between morphological changes in the testes and monthly changes in the differentiation of seminiferous tubules’ spermatogenic cells and review the male reproductive cycle of the Korean greater horseshoe bat (Rhinolophus ferrumequinum korai Kuroda, 1938).

Materials and methods

Experimental animals were collected and examined under the guidelines of the Kyungnam University Institutional Animal Care and Use Committee (KUIAC).

In this study, 40 male Rhinolophus ferrumequinum korai were collected from abandoned mines in Gyeongnam and Jeonnam provinces of South Korea from January 2014 to December 2015 (Table I) using inhalant anesthesia. Monthly collected materials were immersed in 5 mL of 3% glutaraldehyde aqueous solution (4°C, pH 7.4, Milloning’s buffer) and 0.01 mL of RNA stabilization solution (Figure 1). To examine morphological changes of the testes and the stage of differentiation of the seminiferous epithelium according to monthly changes, testis tissues extracted from the epididymis were soaked in 3% glutaraldehyde aqueous solution (4°C, pH 7.4, Milloning’s buffer) for 24 hours. Tunica albuginea was then removed. After that, testicular tissues were cut to 1–1.5 mm³ in size and pre-fixed in 3% glutaraldehyde aqueous solution (4°C, pH 7.4, Milloning’s buffer) for 24 hours.

Table I. Date examined, locality and number of individuals of the Korean greater horseshoe bat, Rhinolophus ferrumequinum korai, tested in this study (all bats were collected from survey sites of abandoned mines).

| Species                      | Period        | Date examined       | Locality                        | No. of bats |
|------------------------------|---------------|---------------------|---------------------------------|-------------|
| *Rhinolophus ferrumequinum korai* | In hibernation | 25 January 2014     | Gwandeok-ri, Dason-myeon, Tongyeong-si, Gyeongnam | 4           |
|                              |               | 27 January 2014     | Yeoyang-ri, Jinjeon-myeon, Changwon-si, Gyeongnam | 2           |
|                              |               | 20 February 2014    | Gwandeok-ri, Dason-myeon, Tongyeong-si, Gyeongnam | 4           |
|                              | Upon arousal  | 19 March 2014       | Gwandeok-ri, Dason-myeon, Tongyeong-si, Gyeongnam | 5           |
|                              |               | 20 April 1994       | Grandeok-ri, Dason-myeon, Tongyeong-si, Gyeongnam | 2           |
|                              |               | 23 May 2015         | Gyeowol-ri, Woldeung-myeon, Suncheon-si, Jeonnam | 2           |
|                              |               | 24 May 2015         | Yeoyang-ri, Jinjeon-myeon, Changwon-si, Gyeongnam | 2           |
|                              |               | 20 June 2014        | Gyeowol-ri, Woldeung-myeon, Suncheon-si, Jeonnam | 2           |
|                              |               | 23 June 2015        | Gyeowol-ri, Woldeung-myeon, Suncheon-si, Jeonnam | 2           |
|                              |               | 20 July 2015        | Gyeowol-ri, Woldeung-myeon, Suncheon-si, Jeonnam | 2           |
|                              |               | 22 August 2015      | Gyeowol-ri, Woldeung-myeon, Suncheon-si, Jeonnam | 2           |
|                              |               | 20 September 2014   | Gyeowol-ri, Woldeung-myeon, Suncheon-si, Jeonnam | 2           |
|                              |               | 19 September 2015   | Gyeowol-ri, Woldeung-myeon, Suncheon-si, Jeonnam | 1           |
|                              | In hibernation | 18 October 2014     | Yeoyang-ri, Jinjeon-myeon, Changwon-si, Gyeongnam | 2           |
|                              |               | 18 October 2015     | Gyeowol-ri, Woldeung-myeon, Suncheon-si, Jeonnam | 3           |
|                              |               | 21 November 2015    | Chamyeon-ri, Gohyeon-myeon, Namhae-gun, Gyeongnam | 2           |
|                              |               | 19 December 2015    | Chamyeon-ri, Gohyeon-myeon, Namhae-gun, Gyeongnam | 2           |
buffer) for 2 hours. After fixation, tissue slices were washed with the same buffer (4°C, pH 7.4, Milling’s buffer) twice (20 min per wash). They were then post-fixed in 1.33% OsO$_4$ aqueous solution (4°C, pH 7.4, Milloning’s buffer) for 2 hours. These fixed tissue pieces were washed twice with the same buffer (20 min per wash). After washing, tissue pieces were dehydrated with increasing acetone concentration (65, 75, 85, 90, 95, 99 and 100%) followed by embedding with Epon 812 synthetic resin. These embedded tissues were cut to 400 nm thickness using an ultramicrotome (MT-1; Sorvall, Dupont) and stained with 0.5% toluidine blue. The differentiation stage of the seminiferous epithelium was observed with an optical microscope. Subsequently, continuous thin sections of 60–70 nm were obtained. They were double-stained with uranyl acetate solution and lead citrate solution followed by observation with a transmission electron microscope (TEM, H-600, Hitachi) at 75 kV.

**Results**

To investigate periodic changes in the male reproductive cycle of Korean *Rhinolophus ferrumequinum korai*, morphological changes of the testes and the differentiation pattern of the seminiferous epithelium were observed by optical and electron microscopy. Spermatogonia were observed in all seminiferous tubules from January to December (Figure 2A–L)). The periodic pattern of the male reproductive cycle was divided into active and hibernation periods. The following results were obtained.

**Monthly morphological changes of testes**

Morphological traits of the testes of *R. ferrumequinum korai* were significantly different according to month (Figure 1A–L)). The size of testes began to gradually decrease from September (Figure 1L) to March of the following year (Figure 1C). It began to gradually increase from April, the awakening phase (Figure 1D). The testis showed the maximum size in August (Figure 1H).

**Differentiation pattern of the seminiferous epithelium during the active phase**

Spermatocytogenesis occurred throughout April (Figure 2D, 6, 7 and Table II) and May (Figures 2E, 6, 7 and Table II). Spermiogenesis progressed from June (Figures 2F, 3A, 6, 7 and Table II) to September (Figures 2I, 3B, 6, 7 and Table II). The activity of spermatogenesis was the highest in mid-August (Figures 2H, 6, 8). The lumen of the seminiferous tubules was open from April (Figures 2D, 6 and Table II) to mid-October (Figures 2J, 4A (inset), 4B (inset), 6 and Table II).

In the seminiferous tubules, a large number of Ad (dark-type) and Ap (pale-type) spermatogonia (Figure 2D), intermediate-type spermatogonia (Figures 2D, 6), and primary spermatocytes were observed (Figures 2D, 6) in April. In May, seminiferous tubules were wider than those in April. The number of primary spermatocytes in May was greater than that in April (Figure 2E). In June, seminiferous tubules started
Male reproductive cycle of *R. ferrumequinum korai*
to show spermatids, including many primary spermatocytes (Figures 2F, 3A, 6). In July, many sperm were observed in the lumen of many elongated spermatids containing primary spermatocytes (Figure 2G). In August, a few primary spermatocytes, a number of early spermatids and mature spermatids, and numerous spermatozoa were present in the lumen (Figure 2H). In September, there were few Ad spermatogonia, several early spermatids (Figures 2I, 3B), abandoned spermatids from Sertoli cell cytoplasm, and numerous sperm in the lumen (Figures 2I, 3B).

### Differentiation pattern of seminiferous epithelium during hibernation

Upon hibernal onset starting in October, spermatozoa of seminiferous tubules were transferred to the lumen of the seminiferous tubules (Figures 2J, 4A). The pattern of seminiferous tubules was the same as that in October. From this time, the lumen was closed. The lumen of the seminiferous tubules in November and December was also closed. Only Ad-type spermatogonia were observed. Ad, dark type spermatogonium; Ap, pale type spermatogonium; B, B type spermatogonium; Bl, basal lamina; D, diplotene spermatocyte; Es, elongating spermatid; In, intermediate spermatogonium; L, lumen; Lf, lipofuscin; M, meta phase Ms, mature spermatid; P, pachytene spermatocyte; PL-L, pre-leptotene/leptotene spermatocyte; Rs, round spermatid; S, sperm; Se, Sertoli cell; St, spermatid; Z, zygotene spermatogonium. A, January; B, February; C, March; D, April; E, May; F, June; G, July; H, August; I, September; J, October; K, November; L, December.
There was no mature sperm (Figures 2J, 4B (inset)). In some seminiferous tubules, spermatogonia of the Ad type, immature sperm cells, and spermatogonia cells were observed (Figure 4A (inset)). For the first time in this period, numerous immature sperm cells were predated as part of the phagocytosis process of Sertoli cells (Figure 4A). In addition, numerous lipofuscin granules were scattered within the cytoplasm of Sertoli cells (Figure 4B) and the lumen of the seminiferous tubules was open (Figures...
Figure 5. Optical and electron micrographs showing the phagocytosis process of Sertoli cells in the seminiferous tubules in November. The seminiferous tubules in November, like those in October, were phagocytized to a number of immature spermatids as part of the phagocytosis process of Sertoli cells (A, B). From this point on, the lumen was closed (A, inset; B, inset). Lipofuscin was scattered within the cytoplasm of Sertoli cells (A, B). Ad, dark type spermatogonium; Lf, Lipofuscin; N, nucleus; Pg, phagosome; Se, Sertoli cell; Spt, sperm tail.

Figure 6. Differentiation stage and periodicity of the seminiferous epithelium according to month. Spermatocytogenesis was in April and May while the process of spermiogenesis was from June to September. The lumen of seminiferous tubules was open from April to October. It was closed from November to March of the following year. Ad, dark-type spermatogonium; Ap, pale-type spermatogonium; B, B-type spermatogonium; D, diplotene spermatocyte; DK, diakinesis; Es, elongating spermatid; In, intermediate spermatogonium; M, metaphase; M/A, metaphase and anaphase of the meiotic cells (meiosis I); Ms, mature spermatid; P, pachytene spermatocyte; PL/L, pre-leptotene/leptotene spermatocyte; Rs, round spermatid; S, sperm; Z, zygotene spermatogonium; □, immature round spermatids.
In November, seminiferous tubules were observed in immature sperm cells (Figure 5A, 5B) in the same manner as in October. Numerous immature sperm cells were predated as part of the phagocytosis process of Sertoli cells (Figure 5A, B). The lumen of seminiferous tubules was closed for the first time (Figures 2K, 5A (inset), 5B (inset)). These seminiferous tubules were also closed in December (Figure 2A), February (Figure 2B), and March (Figure 2C). Only Ad-type spermatogonia and Sertoli cells were present in these seminiferous tubules (Figure 2A–C, L). Many lipofuscin granules were scattered in the cytoplasm of Sertoli cells (Figure 4A, B).

Correlation between differentiation of seminiferous epithelium and monthly temperature changes

According to this study, the size of testes (Figure 1 (A–L)) and the stage of differentiation of seminiferous epithelium were remarkably different according to month (Figure 2(A–L)). These differences were also evident according to annual and monthly temperature changes (Table III). The average annual temperatures in Changwon-si, Tongyeong-si, Namhae-gun in Gyeongnam, and Suncheon-si in Jeonnam in 1991 were 14.6, 14.7, 13.9 and 12.6°C, respectively. The average temperature in these three regions was 14.4°C. In 2015, average temperatures at Changwon-si, Tongyeong-si and Namhae-gun were 14.6, 14.8 and 14.7°C, respectively. The average temperature of these three regions was 14.7°C. It was 13.2°C at Suncheon of Jeollanamdo. When temperatures at Changwon-si, Tongyeong-si, Namhae-gun and Suncheon in April (the spermatogenesis initiation period) were compared between 1991 and 2015, the difference in temperature was about 0.4°C (1991: Changwon, 13.8°C; Tongyeong, 13.1°C; Namhae, 12.8°C; average, 13.2°C; 2015: Changwon, 13.9°C; Tongyeong, 13.2°C; Namhae, 13.7°C; average, 13.6°C). Overall, changes in annual temperature and monthly temperature between 2015 and 1991 were 0.3 to 0.4°C (April for spermatogenesis).

**Discussion**

Spermatogenesis of mammals is a series of complicated and elaborate processes involving maturation and differentiation of diploid (2n) spermatogonia into highly differentiated haploid (n) germ cells (França et al. 1999; Paula et al. 1999; Calvo et al. 2000, 2001). Differentiation processes of spermatogenesis involve many sudden changes in the cell. Such changes have been used for biochemistry and gene regulation studies as well as the identification and classification of ultrastructural changes of sperm (Mori et al. 1991; Jeoung et al. 2006). In addition, the differentiation process of the seminiferous epithelium affects the reproductive cycle according to abiotic factors such as temperature and photoperiod (Heideman et al. 1992; Mello et al. 2009).

Although information on the duration of the seminiferous epithelium cycle is well known for a large number of bats (Beguelini et al. 2012a), there has been no report on the extinguishment of immature spermatids by phagocytosis of Sertoli cells during the annual cycle of the seminiferous epithelium except for the male reproductive type of *R. ferrumequinum*.
Table III. Comparison of temperature changes from the nearest meteorological stations at the same survey sites between 1991–1992 and 2014–2015 (Korea Meteorological Administration 1991, 1992, 2014, 2015).

| Year | Locality | Air temperature (average, °C) |
|------|----------|-------------------------------|
|      | Gyeongsang-namdo | Jeolla-namdo | Gyeongsang-namdo | Jeolla-namdo | Gyeongsang-namdo | Jeolla-namdo | Gyeongsang-namdo | Jeolla-namdo |
|      | Ms (†) Cm (=Ty) Nh Sc | Ms (†) Cm (=Ty) Nh Sc | (‡) Ms (=Cw) Ty Nh (♣) Sc | (‡) Ms (=Cw) Ty Nh (♣) Sc |
| Month |         |         |         |         |         |         |         |         |
| 1     | 2.8     | 3.0     | 2.9     | 0.1     | 4.3     | 4.5     | 3.6     | 1.3     |
| 2     | 3.1     | 3.2     | 2.1     | 0.3     | 4.4     | 4.5     | 3.8     | 1.0     |
| 3     | 8.2     | 1.9     | 7.0     | 6.6     | 9.3     | 9.0     | 8.3     | 7.3     |
| 4     | 13.8    | 13.1    | 12.8    | 12.4    | 14.0    | 13.5    | 13.5    | 12.0    |
| 5     | 17.7    | 16.8    | 17.2    | 16.8    | 17.8    | 16.9    | 17.3    | 15.9    |
| 6     | 22.6    | 21.3    | 22.6    | 22.3    | 20.6    | 19.9    | 20.2    | 20.4    |
| 7     | 24.9    | 23.9    | 25.1    | 25.0    | 25.6    | 24.3    | 25.3    | 25.3    |
| 8     | 24.7    | 24.0    | 24.2    | 24.5    | 25.9    | 25.4    | 25.0    | 25.3    |
| 9     | 23.0    | 22.6    | 22.2    | 21.0    | 23.0    | 22.8    | 22.0    | 22.0    |
| 10    | 17.1    | 16.7    | 15.9    | 13.0    | 16.6    | 16.6    | 15.6    | 13.0    |
| 11    | 10.2    | 16.7    | 9.5     | 6.6     | 10.0    | 10.6    | 9.3     | 6.4     |
| 12    | 6.5     | 6.9     | 5.6     | 2.9     | 6.0     | 6.6     | 5.9     | 3.0     |
| Year average | 14.6 | 14.7 | 13.9 | 12.6 | 14.8 | 14.6 | 14.1 | 11.1 |

Due to administrative district reform, Masan City was integrated into Changwon City in July 2010, while Chungmu City was integrated into Tongyeong City in January 1995.

(1) Annual climatological report (1991). Korea Meteorological Administration Seoul, Republic of Korea. pp. 84–193.

(2) Annual climatological report (1992). Korea Meteorological Administration Seoul, Republic of Korea. pp. 73–166.

(3) Annual climatological report (2014). Korea Meteorological Administration Seoul, Republic of Korea. pp. 84–193.

(4) Annual climatological report (2015). Korea Meteorological Administration Seoul, Republic of Korea. pp. 117–267.

(†) Chungmu Weather Station name was changed from Chungmu (162) to Tongyeong (162) in January of 1995 due to administrative recomposition.

(‡) The Masan Weather Station name was changed from Masan (155) to Changwon (155) in July of 2010 due to administrative recomposition.

(♣) The Suncheon Weather Station number was changed from 256 to 174 in April of 2011.

Cm, Chungmu-si; Cw, Changwon-si; Ms, Masan-si; Nh, Namhae-gun; Sc, Suncheon-si; Ty, Tongyeong-si.
The timing and duration of the spermatogenesis process in bats are similar to each other (Kurohmaru et al. 2002; Kang & Lee 2004; Beguelini et al. 2012a,b, 2013, 2014; Bueno et al. 2014). However, hibernating bat species have unique reproductive patterns. According to the present study, the size of testes began to gradually increase from April, the beginning of spermatogenesis (Figure 1D). In August, the size of testicles was maximized (Figure 1H). It then gradually decreased from September (Figure 1I) to March (Figure 1C), the end of hibernation. In addition, diameter changes of seminiferous tubules showed a tendency almost the same as that of testis size (Figure 1A–C, L). Diameter change is consistent with spermatogenesis. The maximum activity of spermatogenesis is known to be related to the diameter of the largest seminiferous tubules and the maximum testicle weight (Racey 1974; Racey & Tarn 1974; Gustafson 1976). Sperm production is directly related to the size of testes (Amann 1970; França & Russell 1998; Bedford 2008). Although body temperature does not inhibit sperm maturation in epididymis fluid, it has a significant effect on sperm viability and sperm maturation in storage capacity and cauda epididymis (Bedford 2008).

According to some authors, lower temperature of the epididymis makes sperm maturation and storage easier (Foldesy & Bedford 1982; Djakiew & Cardullo 1986; Jolly & Blackshaw 1988).

Considering cell structures of the seminiferous epithelium according to month (Figures 2–6) and the annual cycle of the seminiferous epithelium (Table II and Figures 6–8) shown in this study, spermatogenesis began in April (Figure 2D) and ended in September (Figure 2I). Our results confirm that the male reproductive pattern of Korean R. ferrumequinum korai is the “Pipistrellus pattern”, according to Lee et al. (1993), because spermatogenesis does not occur during the mating period or hibernation period. In particular, spermatocytogenesis occurred throughout April (Figure 2D) and May (Figure 2E) while spermiogenesis occurred from June (Figure 2F) to September (Figure 2I). The activity of spermatogenesis was the highest in mid-August (Figure 2H). In addition, the lumen of seminiferous tubules was open from mid-April (Figure 2D) to mid-October (Figure 2J). It was closed from November (Figure 2K) to March (Figure 2C) of the following year. The mating period was from September to the beginning of October at the latest. This means that most spermatozoa that underwent the spermatogenesis process in August and September might have migrated to the epididymis. After mating, bats soon go into hibernation, and there is no need for sperm production anymore. Therefore, they can have efficient energy use for long hibernation. In particular, from mid-October (Figure 2J) to mid-November (Figure 2K), immature spermatids in seminiferous tubules during hibernation were completely destroyed by phagocytosis of Sertoli cells (Figures 4A, 5A, 5B). In phagocytosis, more than half of differentiating spermatogenic cells are known to undergo cell apoptosis before spermatogenesis in mammals. They are rapidly and selectively cleared by phagocytosis of Sertoli cells (Lee et al. 1993; Nakanishi &
Shiratsuchi 2004). Inhibition of phagocytosis in living animals will result in a decrease in the number of epididymal spermatozoa, suggesting that phosphatidylserine-mediated phagocytosis of apoptotic cells by Sertoli cells is necessary for efficient production of spermatozoa (Nakanishi & Shiratsuchi 2004). In hibernating bats, this phagocytosis is a preparation stage for new spermatogenesis in the next year. This is called a cleansing period.

Spermatogenesis initiation and duration of some hibernating bats are shown in Table II and Figure 8. There were some differences in the spermatogenesis initiation and duration between species (Figure 8). In the case of R. ferrumequinum korai (Lee et al. 1993) and Miniopterus schreibersi fuliginosus (Kang & Lee 2004), spermatogenesis initiation occurred from May. However, initiation of spermatogenesis in Myotis macrodactylus (Lee & Mōri 2004) and R. ferrumequinum korai in this study started in April.

Notably, spermatogenesis of Korean R. ferrumequinum korai occurred in May (Figure 8 and Table II) in a previous study (Lee et al. 1993). However, it occurred in April in this study (Figures 2D, 7, 8 and Table II). The fact that spermatogenesis initiation took place about a month earlier in the present study (Figure 8 and Table II) suggests that the rising temperature might have affected the timing of spermatogenesis (Table III). Based on yearly and monthly temperature changes shown in Table III, the average temperature in 2015 was 0.3 to 0.4°C higher than that in 1991. It has been reported that abiotic factors such as temperature and photoperiod can affect the reproductive cycle of bats (Heideman et al. 1992; Mello et al. 2009). There is a concern that many species might become endangered if they do not develop new seasonal strategies to adapt to the rising temperature (Bradshaw & Holzapfel 2006).

Although the reproductive cycle of the bat Sturnira lilium might be directly influenced by factors such as temperature and food availability (Bronson 1985; Mello et al. 2009), the timing of the male cycle of tropical sperm-storing bats and onyx hibernating bats seems to be dependent on temperature more than on nutrition. Their spermatogenesis is generally stopped or suppressed in the coldest months (Gopalakrishna & Madhavan 1971; Myers 1977; Krishna & Dominic 1981; Krishna 1985).

In conclusion, the testicular reproductive cycle of R. ferrumequinum korai consists of three main stages. The first is the spermatogenesis stage (from April to September), including spermatocytogenesis (which appears from April to May) and spermiation (from June to September). The second is the phagocytosis stage (from mid-October to mid-November), which is a purification process to prepare for the new spermatogenesis in the following year. This period is called the cleansing period. The third is the dormant stage that is a state of holding only spermatogonia and Sertoli cells. This is an adaptation strategy to promote efficient use of energy for long hibernation. Compared to previous studies (Lee et al. 1993), their spermatogenesis period preceded 1 month earlier. This suggests that temperature increase can affect their reproductive development and spermatogenesis. Even with the apparent morphological features shown in this paper, there is a limitation in that it cannot fully explain the mechanism by which the change of temperature can affect the reproductive cycle of Rhinolophus ferrumequinum korai. Therefore, in addition to electron microscope observation used in this paper, other approaches need to be used in parallel to improve our understanding of the relationship between temperature and reproductive cycle changes. Previous reports have suggested that steroid hormones, receptors and oxidative stresses might influence various aspects of reproduction. Further studies on light and temperature variations by antioxidants using enzymatic assay (Guerrero et al. 2003), chromatography (Guerrero et al. 2004) and expression analysis of steroid receptors (Guerrero et al. 2005, 2009, 2017a) and dosing free radicals with real-time quantitative polymerase chain reaction (PCR) (Guerrero et al. 2017b) are needed in the future.

Disclosure statement

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Geolocation information

Country: Korea, Republic of
Region: Gyeongsangnam-do
City: Changwon-Si
Latitude: 35°11′N (Masan-si)
Longitude: 128°34′E (Masan-si)

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