KOREAN JOURNAL OF ZOOLOGY
Vol. 20, No. 2, 67~76 (1977)

Periodic Changes of the Testis and Ductus Epididymis in Korean Hibernating Bats

Yung Keun Oh
(Dept. of Anatomy, Yonsei University College of Medicine)

韓國產 冬眠 박쥐의 精巢와 副精巢의 週期的인 變化에 관한 硏究

吳 永 根
(延世大 醫大 解剖學敎室)
(Received May 12, 1977)

摘 要

韓國產 冬眠박쥐인 안주애기박쥐(*Vespertilio superans* Thomas)와 관박쥐(*Rhinolphus ferrumequinum Korai* Kurdda)의 精巢와 副精巢의 週期的 變化를 봄으로서 그 組織學的인 所見과 生理的인 현상의 一面인 多眠과의 相互關係를 檢討한 바 그 結果는 다음과 같다.

1. 7月에 잡은 안주애기박쥐의 精子形成 機能은 비록 精母細胞의 減數分裂像은 활발하지만 萎縮되어 있었다. 小數의 精子가 8月 박쥐의 精細管에서 發見되었다.

2. 9月에 채집한 관박쥐의 精子形成 機能은 현저하지 않았으나 많은 數의 精子가 副精巢에 貯藏되어 있었으며 副精巢上皮에는 旁小管腔이 나타나는 것을 볼 수 있었다.

3. 12月에 채집한 관박쥐의 精細管上皮는 組織學的으로 衰退된 양상을 보였다. 그러나 副精巢管上皮는 直方形을 띄우고 極度로 膨大된 內腔에는 巨大한 數의 精子가 殘存해 있었다.

4. 이와 같은 결과는 이들 두 種類의 박쥐의 精巢와 副精巢 週期的으로 變하고 이것이 그들의 越冬現象과 密接한 關係가 있음을 暗示하는 것이라고 생각된다.

INTRODUCTION

It has been demonstrated that reproductive activities of hibernating bats are usually carried out in a certain peculiar way which is closely related to one of their special faects, hibernation. Hartman (1933) and Baker and Bird (1936) have set up a hypothesis that copulation usually occur in the fall but ovulation and fertilization takes place in the spring. This means that the mode of reproduction is quite different from those of other mammals; insemination is not always followed by concurrent fertilization.

On the other hand, Wimsatt (1945) and Stebbings (1965) have reported that copulation can occur not only in the fall but also in the winter and even in the spring. In other words, ejaculated sperms are able to survive in the female reproductive tracts, otherwise stored sperms in the male reproductive tracts are able to persist during the long winter waiting for their given task of fertilization in the spring. Thus a certain possibility of the survival of active sperms in the male reproductive tracts has also been emphasized and such an interesting phenomenon has been supported by many workers (Strelkov, 1962; Racey, 1973; Uchida and Mori, 1972, 1974; Mori and Uchida, 1974). With special regard to the mechanism of fertilization in bats, Uchida and Mori (1972, 1974) have reported extensive electron microscopic findings on the mechanism including the process of spermiogenesis and the acrosomal reaction in the Japanese bats.

Whatever the mechanism by which the sperm of bats can persist in the female or male reproductive tracts for such a tedious hibernation period in a condition of low body temperature, it is of interest to study the possible periodic changes of the spermatogenic epithelium and the excretory ducts in Korean hibernating bats, and to elucidate correlations between the morphological facets and the physiological modalities in the reproduction of bats.

The results obtained in the present study suggest that certain periodic changes of the spermatogenic epithelium and the excretory ducts in Korean hibernating bats seem to be closely related to one of their special physiological facets, hibernation.

MATERIALS AND METHODS

Two species of bats, oriental discoloured bats (*Vespertilio sperans* Thomas) and Korean greater horseshoe bats (*Rhinolophus ferrumequinum korai* Kuroda) were used in the present investigation. The oriental discoloured bats (adult male, body weight- 14gm on the average) were obtained from their natural habitat, the tiledroof of a building in the Eujongboo Middle School, Gyonggi-Do, Eujongboo on July 19 and August 1, 1976 and the Korean greater horseshoe (adult male, body weight-19gm on the average) were obtained from the Chunho Cave at Hosan-Ri, Yosan-Myon, Ikssan-Guan, Chullabuk-Do on September 29 and December 5, 1976.

For the sake of convenience and understanding, the experimental animals obtained at various periods were divided into three phases: (1) active (*Vespertilio superans* Thomas captured in July and August, (2) pre-hibernation (*Rhinolophus ferrumequinum korai* Kuroda captured in September, and (3) hibernating (*Rhinolophus ferrumequinum korai* Kuroda) captured in December.

After bringing the bats to the laboratory, the whole testis and epididymis were fixed in 10% formalin, embedded in paraffin, sectioned at 5μ, and stained in hematoxylin and eosin for histological analysis. Seminiferous tubules and interstitial tissue of the testis and ductus epididymidis of the excretory duct were observed with a binocular microscope.

RESULTS

1. The testis and the excretory ducts of *Vespertilio superans* Thomas in the active phase (Fig. 1-5).

There were slight differences between the histologic findings of the testis of the bats obtained in July(Fig. 1) and those in August. The cell layers of the spermatogenic epithelium of the testis obtained in Augnst seemed more statified than those obtained in July. Large type A spermatogonium had a spherical nucleus with fine chromatin granules and usually one irregularly shaped nucleolus (Fig. 2). These type A cells were more predominent in the August bat. In the spermatocytes, active meiotic cell divisions were distinguishable both in the July (Fig. 3) and August bats but more active figures were observed in the latter. None of the mature sperm and spermatids were found in the lumina of the seminiferous tubules in July bats but a few sperm anchored to the extended cytoplasm of the Sertoli cells were observed in those of the August bat(Fig. 4). In contrast to a certain degree of hypertrophy of the spermatogenic epithelium, the interstitial tissues were slightly decreased in amount in the August bats. The interstitial cells of Leidig were irregular in shape and occurred in isolated clusters. The cytoplasm was strongly acidophilic in the July bats but became less eosinophilic in the August bats. The blood vessels also increased in number. In the excretory ducts of both bats, no remarkable histological differences were observed, although slight increases of the lumenal sizes were generally distinguishable (Fig. 5). However, a small number of the spermatid were occasionally found in the tail of the ductus epididymidis of the August bats.

2. The testis and the excretory ducts of *Rhinolophus ferrumequinum korai* Kuroda in the pre-hibernation phase (Fig. 6-8).

The spermatogenic function of September bats was not remarkable, although the spermatogenic epithelium showed 3 to 4 cell layers which were composed of two major categories of cells (Fig. 6). A unmber of the supporting Sertoli cells were mainly observed in the vicinity of the lumen and they were characterized by a star-shaped conformation in contour (Fig. 7). However, few of the spermatogenic cells were distinguishable in the lumen but almost no mature sperm were found in the hollow space. The interstitial tissue of the testis was small in amount and the interstitial cells had large dense spherical nuclei with centrally located nucleoli. In the excretory ducts, the histological findings were quite different from those of the seminiferous tubules so far as functional facets were cencerned. The ductus epididymidis epsecially contained a vast number of sperm that were formed in concentrated clusters here and there. But some times part of the ductus epididymidis did not contain any sperm at all (Fig. 8).

In the basement membrane portion adjacent to the pseudostratified ciliated columnar epithelium of the ductus epididymidis, there were peculiar hollowed spaces which were mostly spherical in shape. These small para-tubular spaces were found only in the Septemer bats, and especially in its head and body of the epididymis (Fig. 8). Sometimes they seemed to be located within or between the epithelial cells. The spaces were usually vacant but some of them contained various materials icluding red blood corpuscles, pyknotic cell nuclei, unclarified cell debris, and unidentified materials.

3. The testis and the excretory ducts of *Rhinolophus ferrumequinum korai* Kuroda in the hibernating phase (Fig. 9-12).

The histoliogical changes of the testis and the ductus epididymidis were more remarkable in December bats than in September bats. A thick fibrous capsule, the tunica albuginea, seemed to become increased in width and the spermatogenic cell layer of the seminiferous tubule became conversely decreased in size (Fig. 9). Even the early cell lines of spermatocytogenesis were scarce in the periphery of the tubule. However, the sustentacular cells were frequently observed between the spermatogenic epithelial cells but never formed a star-shaped arrangement (Fig. 10). In the interstitial tissues, while findings similar to those of September bats, the differentiation of aboundant blood vessels was distinguishable (Fig. 10). The excretory ducts of the December bat were barely developed with the exception of the ductus epididymidis. The diameter of the ductus was extremely expanded reaching about three and half times that of September bats (Fig. 11). In these highly enlarged luminae, a vast number of mature sperm were observed forming clusters along with a certain amount of secretory substance.

The orientation of the sperm was also characteristic as if they were a shoal of fish following a certain sea current (Fig. 12). The smooth muscle cells in the connective tissue between the ducti were greatly elongated due to the extreme expansion of the luminae (Fig. 12). The epithelial cells of the ductus epididymidis were rectangular in shape and which nuclei showed withered contours along the basal portion of the epithelial cells.

DISCUSSION

It has been reported that the fertilization ability of sperm in the male reproductive tracts is closely related to the estrus period. (Chang, 1965; Miyamoto and Chang 1972). In the hibernating bats that inhabit the north temperate zone where Korea is located, their estrus period, may be expected to be extended in accordance with their hibernation until the next spring. Since Pagenstecher(1859) found live sperm stored in the uterus, a number of researchers have demonstrated that not only the female reproductive tracts but also the male contained a considerable number of sperm during the coital period. (Gilbert and Stebbings, 1958; Strelkov, 1962).

In the present study, the histological findings of the testis and the epididymis of the oriental discoloured bats obtained in July and August showed a distinct inactive feature and such a degenerative change could be explained to be representative of a sort of resting phase of spermatogenesis. However, the auther was not able to rationalize that in spite of general retardation of the testicular function, only spermatocytes were found actively proliferating within the epithelium of the seminiferous tubule. As Racey (1973) pointed out, there is still a possibility that the spermatids can persist in the tail of the ductus epididymidis for seven months or more.

In the September bats, the Korean horseshoe bats, the findings differed considerably from those of the oriental discoloured bats. According to Woon (1977) the Korean greater horseshoe bats usually delivered their young once a year between June and July (gestation period-70 to 84 days). Based on this data, the sperm stored in the ductus epididymidis of the September bats should survive at least by the middle of March later year.

That the peculiar appearance of the para-tubular spaces in the ductus epididymidis occurred only in the ductus epididymidis of the September bats and the rectangular shape of the epithelial cells in spite of extreme expansion of the lumen makes the auther presume that the ductus epididymidis plays a role not only in the storing of the sperm but also in providing their support during the long winter. In fact, Courrier (1921) has reported a spontaneous existence of secretions in the lumen of the ductus epididymidis which might be supplied as nutrients for survival of the sperm. Nakano (1928) has also demonstrated that a considerable amount of glycogen was included in the epithelial cells of the ductus epididymidis of bats. Recently, Racey (1975), on the other hand, has reported that there was a mucous substance on the lumenal surface of the ductus epididymidis which might play an important role in protecting the epithelium against the sperm which were apt to infiltrate as an antigen. Actually he found the sperm within the epithelial cells and this fact has supported the previous results obtained by Austin (1960).

There was one more interesting finding in the present study which was a certain kind of orientation of the sperm stored in the ductus epididymidis of the December bats. Observing a mode of sperm heads in the ductus epididymidis, a certain kind of definite orientation was distinguishable, as if they were following the same sea current. Even in the same lumen of the cross-sectioned ductus, several of the sperm currents were recognizable and these findings indicate that there might be some sort of possibilities of factors influencing the direction of the sperm. Racey (1975) has also demonstrated that when exposed to paraffin-oil the sperm of house bats (*Pipistrellus*) were definitely oriented along the adjacent surface, turning their heads to the paraffin-oil. Although the exact mechanism of such an orientation of sperm has not yet been fully understood, there might be a correlation between the results by Racey (1975) and the sperm current observed in the present study.

The present study has dealt with two kinds of bats. It has been generally known that the reproductive modes differ from species to species of bats. Moreover, the present study has not fully covered all the phases of the reproductive cycle both in the oriental discoloured bats and the Korean greater horseshoe bats. Further studies, therefore, will be needed on the remaining phases including the pre-hibernation and hibernating phases for the oriental discoloured bats, and the active and post-hibernation phases for the Korean greater horseshoe bats as well. These studies have not yet been carried out due to the difficulty of procuring these particular bats during the specified phases.

SUMMARY

The periodic changes of testis and ductus epididymidis in Korean hibernating bats, the oriental discoloured bats (*Vesportilio superans* Thomas) and the Korean greater horseshoe bats (*Rhinolophus ferrumequinum korai* Kuroda) were studied in order to clarify the possibility of correlation between their histological findings and one of physiological facets, hibernation, and the results obtained were as follows:

1. The spermatogenic function of the oriental discoloured bats obtained in July and August was depressed although the spermatocytes showed a considerable cell proliferation. Few mature sperms were observed in the seminiferous tubules of the bat obtained in August.

2. The spermatogenic function of the Korean greater horeshoe bats obtained in

September was not remalkable but a considerable number of sperms were stored in the excretory ducts which were characterized by existence of para-tubular spaces in the ductus epididymidis.

3. The spermatogenic epithelia of the Korean greater horseshoe bats obtained in December showed histologically atrophied figures. However, a vast number of sperm remained in extremely expaned luminae of the ductus epididymidis which epithelial cells were maintained rectangular in shape.

4. These results suggest that there are periodic changes of the spermatogenic epithelia and the excretory ducts, and that those histological changes are closely related to their wintering.

REFRENCES

Austin, C.R., 1960. Fate of spermatozoa in the female genital tract. *J. Reprod. Fert.* **1**: 151~156.

Baker, J.R. and T.F. Bird, 1936. The seasons in a tropical rainforest (New Hebrides): Part 4. Insectivorous bats (Vespertilionidae and Rhinolophidae). *J. Linn. Soc.* (Zool.) **40**: 143~161.

Chang, M.C., 1965. Fertilizing life of ferret sperm in the female tract. *J. Exp. Zool,* **158**: 87~100.

Courrier, R., 1921. Sur la role physiologique des secretions uterine et tubaire chez la chauve-souris nibernante. *C.r. Seanc. Soc. Biol.* **84**: 572~574.

Gilbert, O. and R.E. Stebbing, 1958. Winter roosts of bats in West Suffolk. *Proc. Zool. Soc.* Lond. **131**: 329~333.

Hartman, C.G., 1933. On the survival of spermatozoa in the female genital tract of the bat. *Q. Rev. Biol.* **8**: 185~193.

Miyamoto, M. and M.C. Chang, 1972. Fertilizing life of golden hamster spermatozoa in the female treact. *J. Reprod. Fert.* **31**: 131~134.

Mori, T. and T.A. Uchida, 1974. Electron microscopic analysis of the mechanism of fertilization in Chiroptera II. Engulfment of spermatozoa by epithelial cells of the follopian tube in the Japanese house bat, Pipistrellus abramus. *Zoolgigal Magazine* **83**: 163~179.

Nakanno, O., 1928. Ueber die Verteilung des Glykogens beiden zyklischen Verenderungen in den Geschlechts-organen der Fledermaus und ueber die Nahrungsaufnahme spermien in dem wiblichen Geschlechtswege. *Folio anat. jap.* **6**: 777~828.

Pagenstecher, H.A., 1859. Ueber, 2859. Ueber die Begattung von Vesperugo pipistrellus. *Verh. naturh. med. Ver. Heidelb.* **1**: 194~195.

Racey, P.A., 1975. The prolonged survival of spermatozoa in bats. *In*: The biology of the male gamete by J.G. Duckett and P.A. Racey, Suppl. No. 1 *The Biological Journal of the Linnean Society,* **7**: 385~416.

Stebbings, R.E., 1965. Observations during sixteen years on Winter roosts of bats in West Suffolk. *Proc. Zool. Soc.* Lond. 144: 137~143.

Strelkov, P., 1952. The peculiarities of reproduction in bats (Vespertilionidae) near the northern border of their distribution. *Int. Symp. Meth. Mammal. Invest. Brno.* **1960**: 306~311.

Uchida, T.A. and T. Mori, 1972. Electron microscope studies on the fine structure of germ cells in Chiroptera. I. Spermiogenesis in some bats and notes on its phylogenetic significance. *Sci. Bull. Fac. Agr.*, Kyushu Univ. **26**: 399~418.

Uchida, T.A. and T. Mori, 1974. Electron microscopic analysis of the mechanism of fertilization in Chiroptera. I. Acrosomal reaction and consequence to death of the sperm in the Japanese long-fingered bat, Miniopterus schreibersi fuliginosus. *Sci. Bull. Fac. Agr.*, Kyushu Univ. 28: 177~184.

Wimsatt, W.A., 1945. Note on breeding behaviour, pregnancy and parturition in some vespertilionid bats of the Eastern United States. *J. Mammal.* **26**: 23~33.

Woon, P.H., 1957. *In*: Illustrated encyclopedia of fauna and flora of Korea. Ministry of Education, Korea. pp. 302~305.

ILLUSTRATIONS OF FIGURES

Fig. 1. Testis and epididymis of *Vespertilio superans* (July bat). Inactive spermatogenic epithelium showing thin cellular layer, 450X.

Fig. 2. A seminiferous tubule and interstitial tissue of *Vespertilio superans* (July bat). A type A spermatogonium and several type B spermatogonia rest on the thin basement membrane, 1,000X.

Fig. 3. Spermatocytes showing active meiotic proliferation of *Vespertilio superans* (July bat) and eosinophilic interstitial cells, 450X.

Fig. 4. Many type a cells and Sertoli cells anchoring mature sperm, and interstitial cells in cluster of *Vespertilio superans* (August bat) 1,000X.

Fig. 5. Ductus epididymidis of *Vespertilio superans* (July bat) showing vacant lumen, 450X.

Fig. 6. Thick tunica albuginea and spermatogenic epithelium showing a star-shaped conformation of Sertoli cells of *Rhinolophus ferrumequinum korai*, (September bat), 100X.

Fig. 7. Seminiferous tubules of *Rhinolophus ferrumequinum korai* (September bat) showing a number of type A spermatogonia and Spermatids, and small amount of interstitial tissue, 450X.

Fig. 8. Ductus epididymidis containing a vast number of sperm and para-tubular spaces of *Rhinolophus ferrmequinum korai* (September bat), 100X.

Fig. 9. Seminiferous tubules of *Rhinolophus ferrumequinum korai* (December bat) showing thin cellular layers, and ductus efferentes within the thick tunica albuginea, 40X.

Fig. 10. High power of Fig. 9. none of mature sperm are found, 450X.

Fig. 11. Ductus epididymidis of *Rhinolophus ferrumequinum korai* (December bat) showing a vast number of sperm in the extremely expanded luminae, 100X.

Fig. 12. High power of Fig. 11. showing a shoal of sperm and several highly elongated smooth muscle cells in the connective tissue, 450X.

Fig. 1.

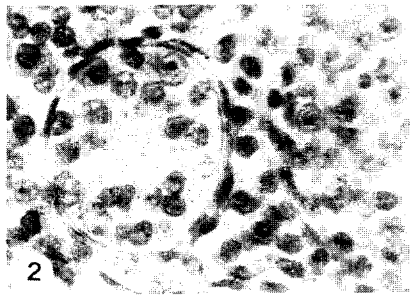

Fig. 2.

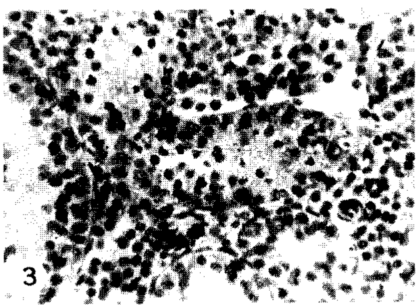

Fig. 3.

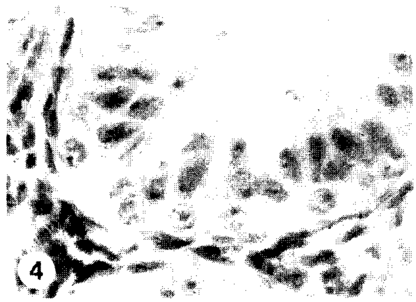

Fig. 4.

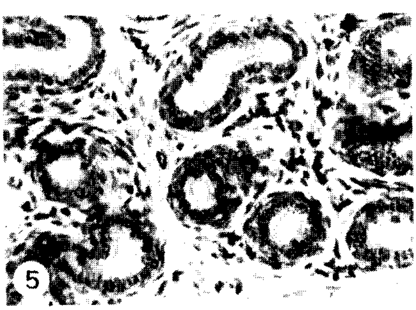

Fig. 5.

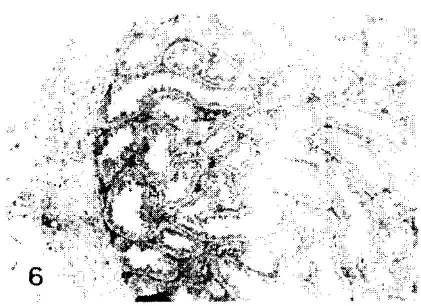

Fig. 6.

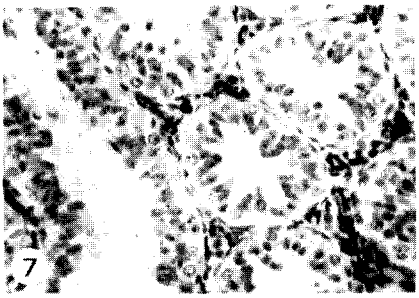

Fig. 7.

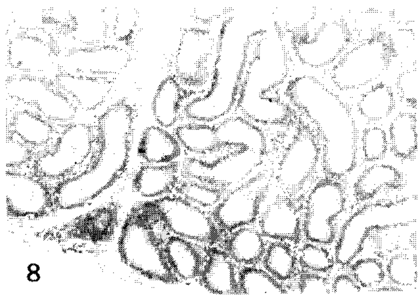

Fig. 8.

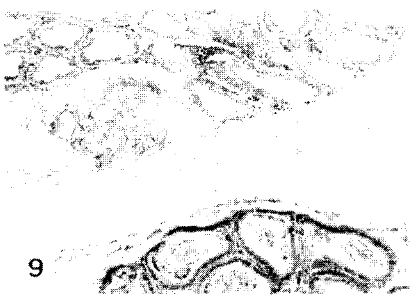

Fig. 9.

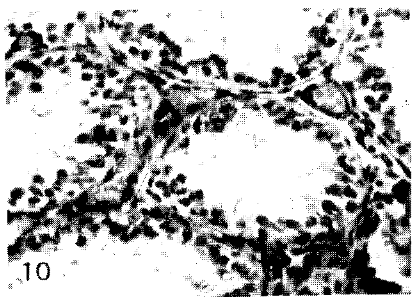

Fig. 10.

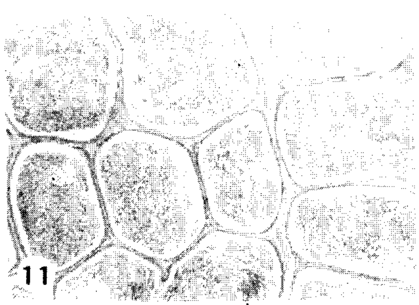

Fig. 11.

Fig. 12.