Morphological changes of cauda epididymis, sperm infiltration into cauda epididymis, sperm storage and sperm disappearance of cauda epididymis in *Rhinolophus ferrumequinum korai* (Chiroptera: Rhinolophidae)

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**Abstract**

In this study to investigate the male reproductive cycle of *Rhinolophus ferrumequinum korai*, we focused on monthly morphological changes of cauda epididymis, sperm infiltration into cauda epididymis, sperm storage and sperm destruction. The caudal epididymal reproductive cycle consisted of two successive purge stages. The first stage of sperm disappearance occurred from April to June. This was a preparation phase for accepting new sperm produced by spermatogenesis, and entailed removing long-stored spermatozoa from the cauda epididymis during the long hibernation period. The second stage of sperm disappearance occurred from July to August, to remove malformed spermatozoa and other residues that were formed during the spermatogenesis process. Therefore, cauda epididymis cleansing lasts about 5 months, from April to August. This period is called the cleansing period. Sperm destruction was achieved by regulating leukocyte phagocytosis and the secretion and uptake by epithelial cells of cauda epididymis. Compared to the author’s previous studies, morphological traits of the cauda epididymis changed 1 month earlier. This suggests that temperature increase can impact reproductive development of the epididymis. Caudal epididymal sperm did not show any sudden morphological change during the long hibernation period after mating. This might be related to a low metabolic rate during hibernation.

**Keywords:** Male reproductive cycle, *Rhinolophus ferrumequinum korai*, caudal epithelial cell, cleansing period

**Introduction**

Mammalian sperm are mature spermatozoa that have undergone physiological and biochemical changes during the transition to cauda epididymis through a continuous differentiation process of seminiferous epithelium (Zaneveld 1982; Voglmayr et al. 1983; Young et al. 1985; Cooper 1986; Hall & Killian 1987). It is well known that proteins and glycosylated proteins synthesized in the caudal epididymis are secreted into the caudal lumen where they can directly interact with the sperm in the lumen (Lea et al. 1978; Moore 1980; Voglmayr et al. 1980). Functions of epithelial cells involved in the secretion process are classified into species and regions. These changes might be due to several factors, including steroid hormones and receptors that are known to influence various aspects of reproduction (Guerriero et al. 2005, 2009; Guerriero 2007), morphological features of spermatogenic cells when they are released from Sertoli cells, or morphological and physiological changes in cauda epididymis. In addition, abiotic factors such as temperature and photoperiodism are important factors that might influence the morphology and the function of spermatozoa (Heideman et al. 1992; Mello et al. 2009; Morais et al. 2013).
The sperm surface of bulls, rats and humans is covered by a layer of Concanavalin A about 40 nm in depth (Baccetti et al. 1978). When radioactive isotopes are used to treat the sperm surface, mature spermatozoa and immature spermatozoa are found to be different from each other (Olson & Hamilton 1978; Voglmayr et al. 1980; Olson & Danzo 1981). In addition, it has been reported that some substances are released from secretory cells of cauda epididymis into the submucosal cavity. They have direct interactions with sperm in the lumen (Lea et al. 1978; Moore 1980; Voglmayr et al. 1980). During the maturation process, several kinds of amorphous proteins, including proteins necessary for sperm’s straight movement, are attached to the plasma membrane (Acott & Hoskins 1981; Klinefeher & Hamilton 1985; Olson et al. 1987). The lipid composition of the plasma membrane can change during the maturation process (Schlegel et al. 1986) as well as the carbohydrate and protein composition (Hall & Killian 1987; Kim et al. 1989). Therefore, the function of the epithelial cells involved in the secretion process varies depending on the species and will have a considerable influence on the morphology and function of the spermatozoa. In addition, sperm entry into the epididymis, sperm destruction, sperm storage and morphological changes in spermatozoa might play important roles in the identification of male reproductive patterns in relation to the functional role of caudal epithelial cells. Therefore, the objective of this study was to determine morphological and numerical changes of spermatozoa from the seminiferous epithelium to cauda epididymis.

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 greater horseshoe bats (Rhinolophus ferrumequinum korai Kuroda, 1938) were collected from abandoned mines in Gyeongnam and Jeonnam provinces for 2 years (from January 2014 to December 2015). They were captured using inhalation anesthetics. Monthly changes in sperm destruction, and inflow and storage of sperm in cauda epididymis through a differentiation process of seminiferous epithelium were determined. To determine the morphology and functional role of caudal epithelial cells, the cauda epididymis was extracted from testes and immersed in 3% glutaraldehyde solution (4°C, pH 7.4, Milloning’s buffer) for 24 hours. After that, the samples were cut to 1–1.5 mm³ size and pre-fixed in a 3% glutaraldehyde aqueous solution (4°C, pH 7.4, Milloning’s buffer) for 2 hours. After fixation, the tissues were washed with the same buffer every 20 min, and tissue slices were post-fixed in 1.33% OsO₄ aqueous solution (4°C, pH 7.4, Milloning’s buffer) for 2 hours. These fixed tissue pieces were washed twice with the same buffer solution every 20 minutes. After washing, the tissue slices were dehydrated in acetone series (65–100%). The tissue slices were embedded in Epon 812, and 400-nm semi-thin sections were cut using an ultramicrotome (MT-1; Sorvall, Dupont). The preparations were stained with 0.5% toluidine blue. The functional role of caudal epithelial cells and the fate of sperm in the lumen of cauda epididymis were observed with an optical microscope. Subsequently, continuous sections with thicknesses of 60–70 nm were obtained, double-stained with uranyl acetate and lead citrate solution, and examined with a transmission electron microscope (TEM, H-600, Hitachi) at 75 kV.

Results

The processes of sperm entry into cauda epididymis, sperm storage, and sperm destruction during the active period were studied.

March experimental group

In the awakening group, the cauda epididymis contained numerous spermatozoa and some secretory cells during mid-March (Figure 1(c)). Compared to the experimental group in January (Figure 1(a)) and February (Figure 1(b)), secretory cells during this period showed secretion activity. The lumen of the cauda epididymis was somewhat turbid due to the presence of these secretory substances.

April experimental group

In April, the diameter of the cauda epididymis was smaller than in March. Sperm were also stored in the lumen (Figure 1(d)). Secretory cells during this period appeared to be more active than in the experimental group in March. The lumen of the cauda epididymis was mixed with amorphous secretory substances (Figures 1(d), 2(a)). In addition, leukocytes penetrated into intercellular spaces of caudal epithelial cells. Many lysosomes were observed in the cytoplasm of epithelial cells (Figure 2(b)).

May experimental group

In May, the diameter of the cauda epididymis tube was smaller than that in April. Very few sperm were
Figure 1. Photomicrograph showing sperm entry, storage and disappearance into cauda epididymis. Numerous spermatozoa were stored in the lumen of cauda epididymis in January (a) and February (b). In particular, there was no change in the shape or number of sperm. Regarding epithelial cell types of cauda epididymis, principal cells and clear cells were visible. Clear cells did not show any secretion activity. Clear cells in the experimental group in March showed some secretion activity. At the same time, the lumen was somewhat turbid. (c) Note that the diameter of the cauda epididymis tubule was smaller than that of the experimental group in April. Leukocytes were infiltrated into the intercellular space of the caudal epithelium (d), especially in the experimental group in April. Note that the diameter of the cauda epididymis tubule was smaller in the experimental group in May (e) than that in the experimental group in April. Very few spermatozoa were stored, especially in the lumen (e). In June, the spermatozoa were not observed in the lumen of cauda epididymis (f). A large number of absorptive vesicles were observed in the cytoplasm of epithelial cells. In the lumen of the cauda epididymis in the experimental group in July, a few spermatozoa were produced as part of the differentiation process of the testes. Immature spermatids infiltrated with these sperm were observed in the lumen (g). With regard to the experimental group in August, new sperm cells made from testes flowed into the cauda epididymis. The lumen was also filled with sperm (h). In September, the lumen was filled with infused sperm (i). In October, lumen of the cauda epididymis was filled with these infused sperm (j). In November and December, the sperm were still stored in the lumen of cauda epididymis (k, l). Cc, clear cell; Ec, epithelial cell; Ims, immature spermatid; L, lumen; Lc, leucocyte; S, sperm; Sm, secretory material.
stored in the lumen (Figures 1(e) and 3(inset)). Stored spermatozoa were destroyed in several parts of the plasma membrane (Figure 3 (arrows)). In addition, the lumen was filled with secretory substances due to vigorous activity of secretory cells in the lumen (Figures 1(e), 3(inset)). Numerous leucocytes were also observed in intercellular spaces of the caudal epithelial cells (Figures 1(e), 2(b)).

June experimental group

Unusually, sperm were not observed in the lumen of the cauda epididymis in June (Figures 1(f), 4

Figure 2. Electron micrograph (April group) showing atypical secretory substances in the lumen of cauda epididymis and leukocytes infiltrating into intercellular space of caudal epithelial cells. The lumen of the cauda epididymis was highly turbid due to secreted materials (a). A number of lysosomes were observed in the cytoplasm of epithelial cells adjacent to the leukocyte infiltration periphery (b). Bc, basal cell; Bl, basal lamina; Ec, epithelial cell; Is, intercellular space; L, lumen; Lc, leucocyte; Lf, lipofuscin; Ly, lysosome; Sh, sperm head; Sm, secretory material; Spt, sperm tail; Stc, stereocilia.

Figure 3. Optical and electron micrograph (May group) showing the appearance of destroyed sperm in the lumen of cauda epididymis. Note that the number of spermatozoa was decreased (inset) and that the sperm plasma membrane was destroyed at various sites (Figure 4, arrows). In addition, the lumen was filled with secretory substances (inset). Ec, epithelial cell; L, lumen; M, mitochondria; N, nucleus; Sh, sperm head; Sm, secretory material; Spt, sperm tail.
A large number of absorptive vesicles were observed in the cytoplasm of the caudal epithelial cells. These absorptive vesicles were filled with substances that appeared to be degradation products (Figure 4).

**July experimental group**

In cauda epididymis in July, a small number of spermatozoa were produced as part of the differentiation process of the seminiferous epithelium of the testis. They were stored in the cauda epididymis. Immature spermatids were also observed in the lumen of the caudal epididymis (Figure 1(g)). In this time period, the caudal epididymal lumen was cloudy due to the activity of secretory cells (Figure 5).

**August experimental group**

During August, more new spermatozoa made from testes entered into the epididymis than in the experimental group in July, and the lumen was full of sperm (Figure 1(h)). Secretory cells were very active during this period. These secretory epithelial cells contained active secretory vesicles in the cytoplasm and secretory substances in the lumen of these vesicles (Figure 6(inset)). In the cytoplasm of secretory cells, many lysosomes and well-developed Golgi complexes were present at the upper surface of the nucleus. Caudal epididymal lumen also looked cleaner than that in the experimental group in July.

**September experimental group**

During this period, the caudal epididymal lumen was filled with these infused sperm (Figures 1(i), 7(inset)). Cauda epithelial cells at this stage showed very active absorption. These cells formed highly developed Golgi complexes on the upper surface of the nucleus. Large and small secreted vesicles were scattered on the right side of the cell. Debris that appeared to be decomposed in the lumen was also found in large and
small vesicles. In the cytoplasm of these absorptive epithelial cells, many lysosomes were observed.

Sperm storage and histology of cauda epididymis during hibernation

October experimental group

Similar to the experimental group in September, the caudal epididymal lumen was also filled with infused sperm (Figures 1(j), 8). A few spermatids showed in the lumen of the cauda epididymis (arrows).

Experimental group of November–February of the following year

Diameters of caudal epididymal tubules of the experimental group in November (Figure 1(k)) and December (Figure 1(l)) were similar to those in January (Figure 1(a)) and February (Figure 1(b)) of the following year. However, in January and February, the experimental group showed secretory cells. Such cells were not present in November or December. In addition, numerous spermatozoa were stored in the lumen of the cauda epididymis in January and February (Figure 1(a,b)).

Discussion

The maturation process for immature spermatozoa involves differentiation within the seminiferous epithelium resulting in fertilization capability. It is caused by infiltration of small amounts of sperm produced by subcellular epithelial cells (Zaneveld 1982; Voglmayr et al. 1983; Cooper 1986). In this case, the time before and after the sperm enters the epididymides is very important to understand the internal environment of spermatogenesis in the testes and to clarify the function of the subcellular epithelial cells that constitute the maturation environment of spermatozoa.

According to this study, almost all spermatozoa newly formed in the testis from July began to be stored in the cauda epididymis. In the mating season (from the beginning of September to the beginning of October to early March of the following year), many spermatozoa were found in the lumen of the cauda epididymis (Figure 1(a–c, h–l)), consistent with results of a previous study (Lee et al. 1993). Spermatozoa exhibited very active motility during the active period. Ligation with cauda epididymis and smearing of semen on the slide were observed. However, sperm motion in the semen of epididymis during the hibernation period was not observed at all (J.H. Lee, unpublished observations on Rhinolophus ferrumequinum korai). This might be an adaptation strategy to efficiently use energy and maintain low metabolic rates during hibernation. On the other hand, Hiraiwa and Uchida (1955) reported that sperm in the cauda epididymis of Pipistrellus abramus can survive from autumn until spring of the following year. They can mate again in early spring, suggesting that the sperm of the cauda epididymis during hibernation has the potential for fertilization. On the other hand, seven out of 10 female spermatozoa of Nyctalus noctula were delivered in May to June, while the spermatozoa of the cauda epididymis were delivered in March when they have fertilizing capacity (Racey 1972). However, post-mating females might show vaginal plug formation (Oh et al. 1983), suggesting that the vaginal plug is not involved in fertilization because they are difficult to mate again (J.H. Lee, unpublished observations on Rhinolophus ferrumequinum korai). In the case of Pipistrellus abramus (Uchida et al. 1988), artificial fertilization with the sperm of cauda epididymis was carried out at the end of March. This means that the sperm does not undergo physiological or
biochemical changes in the female reproductive tract. Therefore, the capacitation concept might have to be reconsidered. On the other hand, results clearly showed that spermatozoa were stored in the cauda epididymis during the hibernation period. They might be involved in fertilization at the time of mating. This was confirmed in the study of Lee et al. (1993) and the current study. During the hibernation period, numerous spermatozoa of the cauda epididymis were observed in the middle of April (Figure 1(d)) and mid-May (Figure 1(e)). They were then completely destroyed in June (Figures 1(f), 3). This process of sperm destruction is a result of regulation of leukocyte phagocytosis and the secretion and uptake of epidermis of the epididymis (Figure 2(a,b)). It might be a process of holding fresh sperm from the testes (Figure 9). After awakening from hibernation, stored spermatozoa in irregular areas are destroyed from April to June to undergo an extinction process (Figure 9(a, step I)). This period can be regarded as the cleansing period as proposed by Lee et al. (1993). Morphological changes with the decreasing number of spermatozoa imply that secretion from the epithelium of the epididymis immunochemically interacted with the surface of spermatozoa in rabbit and cow (Hunter 1969; Barker & Ammann 1971). In addition, differentiation, structure, absorption and secretion functions of the epithelial cells of epididymis as well as the protein composition of adventitious juice are known to be regulated by the testis (Danzo et al. 1977; Moore 1980; Brown et al. 1983; Kim et al. 1989; Lee et al. 1993).

The results of this study revealed the appearance of leukocytes in intercellular space of the caudal epithelial cells in the experimental group in April (Figure 2(b)), suggesting destruction of old spermatozoa (Figures 2(a), 3). Sperm destruction was not only observed from April to June, but also observed in July and August. In addition to newly formed sperm from the testis, immature sperm and malformed sperm were transferred to the cauda epididymis and stored. These abnormal sperm were removed by secretory activity of epithelial cells of the cauda epididymis. As part of the pinocytosis process, absorptive epithelial cells process these degradation products (Figure 9(b, step II)). The purification process might occur to keep the environment of the lumen of the caudal epididymis clean so that new and high-quality sperm can come from the testicles.
Therefore, the reproductive cycle of the cauda epididymis in hibernating Korean *R. ferrumequinum korai* is comprised of the following sequential processes. The reproductive cycle of the cauda epididymis during the activity (arousal) period consisted of two successive stages of sperm destruction in the cauda epididymis (Figure 9). The first stage of sperm destruction appeared from April to June. This was in preparation for accepting new sperm produced by spermatogenesis, by eliminating old spermatozoa stored in the long hibernation period. The second stage of sperm destruction occurred from July to August. This was done by removing immature sperm, malformed spermatozoa and other residues that had flowed into the testis from the spermatogenesis process. This was also a purification process to prepare for the mating period by clearing the lumen of cauda epididymis and retaining mature spermatozoa. Therefore, the period of cauda epididymis cleansing was about 5 months (from April to August). This period is called the cleansing period. Compared to results of previous studies (Lee et al. 1993), the caudal epididymal reproductive cycle occurred about 1 month earlier in this study. An increase in temperature might have affected reproductive development and spermatogenesis. However, it is currently unclear whether a change of temperature can directly affect the reproductive cycle. Oxidative stress, which largely controls endocrine events in reproduction, is one possible explanation (Guerriero et al. 2003, 2004, 2014, 2017a,b).

Sperm destruction was induced by phagocytosis of leukocytes. It was regulated by both secretion and reuptake by caudal epithelial cells. On the other hand, mature sperm storage in cauda epididymis for mating period.

**Figure 8.** An electron micrograph (October group) showing the number of spermatozoa stored in the lumen of cauda epididymis. There were many sperm but very few immature sperm (arrows). L, lumen; M, mitochondria; N, nucleus.

**Figure 9.** Periodic patterns of sperm infestation, sperm storage and sperm destruction in cauda epididymis of hibernating Korean bat *Rhinolophus ferrumequinum korai* according to month. The reproductive cycle of the epididymis had two successive stages of purification and sperm destruction in cauda epididymis during the activity (arousal) period. (a) The first stage of sperm destruction was seen from April to June, which was a preparation stage for removing old spermatozoa during long hibernation so that it would be ready to accept new spermatozoa from spermatogenesis (step I). (b) The second stage of sperm destruction occurred from July to August. This involved retaining mature sperm while removing malformed sperm and other residues from the cauda epididymis. It was also a purification process as a preparatory step to get ready for the mating season (step II). Therefore, the purification period of cauda epididymis lasted about 5 months (from April to August). This period is called the cleansing period. (c) Mature sperm storage in cauda epididymis for mating period. (d) Sperm stored during the hibernation period after completing the mating period.
hand, during the long hibernation period (from mid-October to early March of the following year) after mating, spermatozoa in the cauda epididymis did not show any sudden morphological change. This might be associated with the low metabolic rate that occurs during hibernation.

Disclosure statement

No potential conflict of interest was reported by the author.

References

Acott TS, Hoskins DD. 1981. Bovine sperm forward motility proteins: Binding toepididymal spermatozoa. Biology of Reproduction. 24:234–240. DOI: 10.1095/biolreprod24.2.234.

Baccetti B, Bigiardi E, Burrini AG. 1978. The cell surface during mammalian spermiogenesis. Developmental Biology. 63:187–196. DOI: 10.1016/0012-1606(78)90124-0.

Barker LDS, Ammann RP. 1971. Epididymal physiology. Immunofluorescent analyses of epithelial secretion and absorption and bovine sperm maturation. Journal of Reproduction and Fertility. 26:319–332.

Brown DV, Amann RP, Wagle LM. 1983. Influence of rate testis fluid on the metabolism of testosterone by cultured principal cells isolated from the proximal or distal caput of the rat epididymis. Biology of Reproduction. 28:1257–1268. DOI: 10.1095/biolreprod28.5.1257.

Cooper TG. 1986. The epididymis sperm maturation and fertilization. New York: Springer-Velag.117–230. DOI: 10.1007/978-3-642-71471-9_1.

Danzo BJ, Cooper TG, Orehin-Crist MC. 1977. Androgen binding protein (SBP) in fluids collected from the rate testis and cauda epididymis of sexually mature and immature rabbit and observation morphological change in the epididymis following ligation of the ductuli efferent. Biology of Reproduction. 17:64–73. DOI: 10.1095/biolreprod17.1.64.

Guerriero G. 2007. Seasonal steroids variations and maturity stages in the female chub, Leuciscus cephalus L. (Pisces, Cyprinidae). Italian Journal of Zoology 74:317–324 DOI: 10.1080/11250000701448262.

Guerriero G, Bruno MV, Labar S, Bianchi AR, Trochia S, Rabbioto D, Palombo G, Abdel-Gawad FK, De Maio A 2017a. Frog (Pelophylax bergeri, Günther 1986) endocrine disruption assessment: Characterization and role of skin polyp (ADP-ribose) polymerases. Environmental Science and Pollution Research 1–11. DOI: 10.1007/s11356-017-0395-2

Guerriero G, D’Errico G, Di Giaino R, Rabbioto D, Olarewaju OS, Ciarcia G. 2017b. Reactive oxygen species and glutathione antioxidants in the testis of the soil biosentinel Podarcis sicula (Rafinesque 1810). Environmental Science and Pollution Research:1-11. DOI: 10.1007/s11356-017-0098-8.

Guerriero G, Di Finizio A, Ciarcia G. 2003. Oxidative defenses in the sea bass, Dicentrarchus labrax. In: Dunn JF, Swartz HM, editors. Oxygen transport to tissue XXIV. Boston, MA: Springer. pp. 681–688.

Guerriero G, Ferro R, Russo GL, Ciarcia G. 2004. Vitamin E in early stages of sea bass (Dicentrarchus labrax) development. Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology 138:435–439 doi: 10.1016/j.cbpa.2004.06.003.

Guerriero G, Prins GS, Birch L, Ciarcia G. 2005. Neurodistribution of androgen receptor immunoreactivity in the male frog, Rana esculenta. Annals of the New York Academy of Sciences 1040:332–336. DOI: 10.1196/annals.1327.054.

Guerriero G, Roselli CE, Ciarcia G. 2009. The amphibian (Rana esculenta) brain progesterone receptor: Relationship to plasma steroids and vitellogenic cycle during the gonadal recovery phase. Annals of the New York Academy of Sciences 1163:407–409. DOI: 10.1111/j.1749-6632.2009.04438.x.

Guerriero G, Trochia S, Abdel-Gawad FK, Ciarcia G. 2014. Roles of reactive oxygen species in the spermatogenesis regulation. Frontiers in Endocrinology 5:56. DOI: 10.3389/fendo.2014.00056.

Hall JC, Killian GJ. 1987. Changes in rat sperm membrane glycosidase activities and carbohydrate and proteins associated with epididymal transit. Biology of Reproduction. 36:709–718. DOI: 10.1095/biolreprod36.3.709.

Heideman PD, Deoraj P, Bronson FH. 1992. Seasonal reproduction of a tropical bat, Anoura geoffroyi, in relation to photoperiod. Journal of Reproduction and Fertility 96:765–773. DOI: 10.1530/jrf.0.0960765.

Hiraiwa YK, Uchida TA. 1955. Fertilization in the bat, Pipistrellus abramus (Temminck). On the properties of semen stored in the uterus. Science Bulletin of the Faculty of Agriculture Kyushu University. 15:255–267.

Hunter AG. 1969. Differentiation of rabbit sperm antigens from those seminal plasma. Journal of Reproduction and Fertility. 20: 413–418. DOI: 10.1530/jrf.0.0200413.

Kim MK, Yoon HS, Choi KW, Yoon YD. 1989. Lumination of epididymis and electrophoretic patterns of proteins in epididymal fluid during sexual maturation in mouse. Korean Journal of Zoology. 32:264–274.

Klinefelter GR, Hamilton DW. 1985. Synthesis and secretion of proteins by perfused epididymal tubules and association of secreted proteins with spermatozoa. Biology of Reproduction. 33:1017–1027. DOI: 10.1095/biolreprod33.4.1017.

Lee OA, Petrusz P, French FS. 1978. Purification and localization of acidic epididymal glycoprotein (AEG): A sperm coating protein secreted by the rat epididymis. International Journal of Andrology Supplement. 2:592–607. DOI: 10.1111/j.1365-2605.1978.tb0511.x.

Lee JH, Son SW, Möri T, Shiraiishi S. 1993. Studies on the reproductive pattern in the male of Korean greater horseshoe bat, Rhinolophus ferrumequinum korei. Histological changes of cauda epididymis by sperm entrance, storage and disappearance. Korean Journal of Zoology. 36:51–66.

Mello MAR, Kalko EKV, Silva WR. 2009. Ambient temperature is more important than food availability in explaining reproductive timing of the bat Stenura lilium (Mammalia: Chiroptera) in a montane Atlantic forest. Canadian Journal of Zoology 87:239–245. DOI: 10.1139/Z09-010.

Moore HDM. 1980. Localization of specific glycoproteins secreted by the rabbit and hamster epididymis. Biology of Reproduction 705–718. DOI: 10.1093/biolreprod.22.3.705.22.

Morais DB, Paula TAR, Barros MS, Balarini MK, Freitas MB, Matta SLP. 2013. Stages and duration of the seminiferous epithelium cycle in the bat Stenura lilium. Journal of Anatomy. 222:372–379. DOI: 10.1111/joa.12016.

Oh YK, Möri T, Uchida TA. 1983. Studies on the vaginal plug of the Japanese greater horseshoe bat, Rhinolophus ferrumequinum Nippon. Journal of Reproduction and Fertility 68:365–369. DOI: 10.1530/jrf.0.0680365.
Olson GE, Danzo BJ. 1981. Surface changes in rat spermatozoa during epididymal transit. Biology of Reproduction 24:431–443. DOI: 10.1095/biolreprod24.2.431.

Olson GE, Hamilton DW. 1978. Characterization of the surface glycoproteins of rat spermatozoa. Biology of Reproduction 19:26–35. DOI: 10.1095/biolreprod19.1.26.

Olson GE, Lifsics MR, Winfrey VP, Rifkin JM. 1987. Modification of the rat sperm flagellar plasma membrane during maturation in the epididymis. Journal of Andrology 8:129–147. DOI: 10.1002/j.1939-4640.1987.tb02424.x.

Racey PA. 1972. Viability of spermatozoa after prolonged storage in the epididymis. Journal of Reproduction and Fertility. 28:309–311. DOI: 10.1530/jrf.0.0280309.

Schlegel RA, Hammerstedt RH, Cofer GP, Kozarsky K, Freidus D, Williamson P. 1986. Changes in the organization of the lipid bilayer of the plasma membrane during spermatogenesis and epididymal maturation. Biology of Reproduction. 34:379–391. DOI: 10.1095/biolreprod34.2.379.

Uchida TA, Möri T, Son SW. 1988. Delayed capacitation of sperm in the Japanese house bat, P. abramus. Journal of the Mammalogical Society of Japan. 13:1–10.

Voglmayr JK, Fairbanks G, Jackowitz MA, Colella JR. 1980. Post-testicular developmental changes in the ram sperm cell surface and their relationship to luminal fluid proteins of the reproductive tract. Biology of Reproduction 22:655–667. DOI: 10.1093/biolreprod22.3.655.

Voglmayr JK, Fairbanks G, Lewis KG. 1983. Surface glycoprotein changes in ram spermatozoa during epididymal maturation. Biology of Reproduction. 29: 767–777. DOI: 10.1095/biolreprod29.3.767.

Young LG, Hinton BT, Gould KG. 1985. Surface changes in chimpanzee sperm during epididymal transit. Biology of Reproduction. 32:339–412. DOI: 10.1095/biolreprod32.2.399.

Zaneveld LDJ. 1982. Biochemistry of mammalian reproduction. New York, NY: John Wiley and Sons Inc. 37–67.