

## Chapter 3

# Specific Mechanism of Sperm Storage in Avian Oviducts

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**Abstract** The capability for sperm storage in the female genital tract is frequently observed in vertebrates as well as in invertebrates. Because of the presence of a system that maintains the ejaculated sperm alive in the female reproductive tract in a variety of animals, including insects, fish, amphibians, reptiles, birds, and in mammals, this strategy appears to be advantageous for animal reproduction. Although the occurrence and physiological reasons for sperm storage have been reported extensively in many species, the mechanism for sperm storage in the female reproductive tract has been poorly understood until recently. In this chapter, we report our recent findings on the mechanism of sperm storage in avian oviducts, especially data obtained from the Japanese quail (*Coturnix japonica*), as an experimental model. Because sperm storage in birds occurs at body temperature (i.e. 41 °C), elucidation of the mechanism of sperm maintenance in the avian oviduct may open up new avenues for the development of novel strategies for sperm storage in vitro without cryopreservation.

**Keywords** Birds • Female reproductive tract • Sperm • Sperm storage tubules

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### 3.1 Introduction

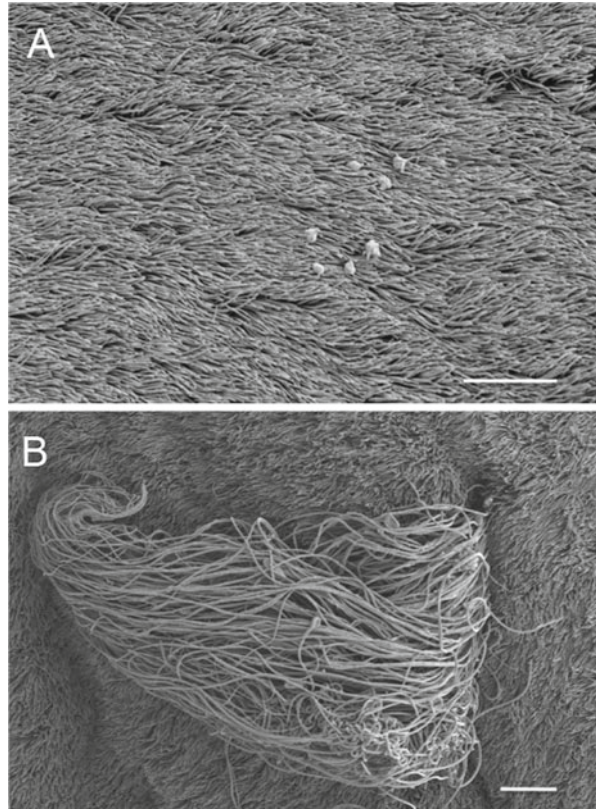
The timing of ovulation is known to be regulated by endocrine factors such as luteinizing hormone and progesterone in higher vertebrates, but the time of insemination into the female reproductive tract by natural mating or artificial insemination does not always synchronize with that of ovulation. To achieve efficient fertilization, sperm need to migrate to the site of fertilization when the ovulated oocytes are there. To increase the chance of fertilization, female animals frequently store sperm in their reproductive tract, and thus sperm storage works as a natural mechanism ensuring sperm encounter the ovulated oocytes at the right time and right place. For instance, some bat species mate in autumn, but fertilization does not take place immediately: the bats store the sperm in the oviduct for 5 months and fertilize them in the spring of the following year (Holt 2011). This phenomenon also optimizes the timing of the birth of their offspring until a suitable season for nursing arrives. Some reptiles, such as turtles, snakes, and lizards, have obvious potential for sperm storage in the oviduct for an extremely long period (maximum, 7 years). This long-term storage appears to work as an insurance against not finding mating partners in some breeding seasons (Holt and Lloyd 2010).

Because of the presence of specialized simple tubular invaginations in the oviduct, once ejaculated sperm have entered the female reproductive tract, they can survive up to 2–15 weeks in domestic birds, including chickens, turkeys, quail, and ducks, for various periods depending on the species (Bakst et al. 1994; Bakst 2011) in contrast to the relatively short lifespan in mammalian spermatozoa (i.e., several days). These specialized structures are generally referred to as sperm storage tubules (SST). SSTs are located in the uterovaginal junction (UVJ) and in the infundibulum, although the primary storage site for sperm is the SST in the UVJ (Burke and Ogasawara 1969; Brillard 1993). The spermatozoa are transported to the infundibulum, which is the site of fertilization and also serves as a secondary sperm storage site (Bakst 1981; Schindler et al. 1967). Although extensive investigations concerning the function of the SST in birds have been performed since its discovery in the 1960s by means of ultrastructural analysis (Bohr et al. 1964; Schuppert et al. 1984; Van Krey et al. 1967), the specific mechanisms involved in sperm uptake into the SST, sperm maintenance within it, and controlled sperm release from it remain to be elucidated. In this chapter, we report our recent findings on the mechanism of sperm storage in the avian oviduct, especially data obtained from the Japanese quail (*Coturnix japonica*), as an experimental model.

### 3.2 Sperm Release from the SST Is a Regulated Event in Birds

There are several reports indicating that sperm release from the SST is not regulated, but occurs in response to the mechanical pressures of a passing ovum, because no contractile elements associated with the SST were found (Van Krey et al. 1967; Tingari and

**Fig. 3.1** Ultrastructural observation of the uterovaginal junction (UVJ) surface treated with progesterone. After mating, the animals were injected with vehicle alone (a) or 0.8  $\mu\text{g}/\text{ml}$  progesterone (b). The UVJ was isolated 1 h after the injection, and the area of the entrance to the sperm storage tubules (SST) was observed by scanning electron microscopy. A representative photograph from those obtained from three different birds is shown. *Bar* = 10  $\mu\text{m}$

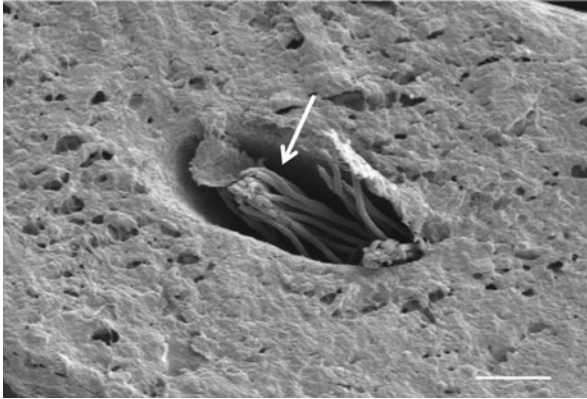


Lake 1973). In contrast, there is conflicting evidence showing that egress of the spermatozoa is regulated because resident spermatozoa were discharged from the SST close to the times of ovulation and oviposition (Bohr et al. 1964). To examine whether sperm release from the SST is regulated during the ovulatory cycle, female birds were mated 12 h after oviposition, and the SST in the UVJ at 2 or 13 h after mating (corresponding to a time 14 or 25 h after oviposition, respectively) was observed. The percentage of the SST containing sperm at 14 h after oviposition was high (i.e., approximately 50–60 %) and significantly decreased to approximately 40 % at 25 h. Also, a bundle of sperm extruding into the lumen of the UVJ from the SST was frequently seen at 20 h after oviposition, although no such sperm were observed at 8, 14, or 25 h. To test whether hormonal stimulation causes sperm release from the SST, the birds were injected with various steroid hormones and the SST filling rate was calculated. As a result, the percentage of the SST with sperm was only significantly decreased when the animals were treated with more than 0.8  $\mu\text{g}/\text{ml}$  progesterone compared to that of the control birds, which were injected with a vehicle alone. Scanning electron microscopic observation revealed that the SST shrank in response to the injection of progesterone, and a bundle of the sperm tail extruded from the SST was observed (Fig. 3.1). This morphological change showed SST squeezed out the resident sperm into the lumen of the oviduct.

These results demonstrated that the release of the sperm from the SST is a regulated event during the ovulatory cycle, and progesterone acts as a sperm-releasing factor in birds (Ito et al. 2011). If the resident sperm are released from the SST without any regulation, most of the sperm ascending the oviduct may be trapped by the descending egg. It is reasonable to suppose that the sperm release from the SST is stimulated by progesterone because there is at least a 5-h grace period before the next ovulation and sperm released from the SST can reach the site of fertilization without hindrance from the descending egg. This process may be supported by a lubricant effect of cuticle materials, because the release of cuticle materials from the epithelial cells of the UVJ is also stimulated by progesterone injection (Ito et al. 2011).

### 3.3 Sperm Maintenance in the SST

Although the period of sperm storage is different in different species, once they have mated, female birds are able to produce fertilized eggs without repeated mating after a long period (e.g., maximum 3 months in the turkey hen). This is possible because these female birds store the resident sperm in the SST of the UVJ after mating, and the resident sperm are thought to be discharged from the SST by the stimulation of progesterone in each ovulatory cycle. This phenomenon suggests that the resident sperm can survive in the SST for extended periods at body temperature in birds (i.e., 41 °C). This surprising phenomenon was discovered more than half a century ago, but we currently know little about the mechanism that supports such long-term maintenance of the resident sperm in the SST of the female reproductive tract in birds. To clarify the SST functions, we first observed the resident sperm in the lumen of the SST by electron microscopy. It is reported that the sperm at the uterotubal junction (UTJ) in the bovine oviduct binds to the surface of epithelial cells and that this binding ensures the tethering of the sperm at the UTJ until the time of ovulation (Hunter 2008; Suarez 2010). In contrast to the situation in mammalian species, the resident sperm seems to be free from the epithelial cells of the SST in the quail oviduct (Fig. 3.2). This finding led us to hypothesize that unknown materials in the lumen of the SST may affect sperm mobility. To confirm this hypothesis, we incubated the ejaculated sperm in the presence or absence of prepared UVJ extracts. The flagellar movement of the sperm was recorded using a high-speed camera. When the sperm were incubated in the absence of the UVJ extracts, a vigorous flagellar movement was observed (Fig. 3.3a). However, in the presence of the UVJ extracts, we found that the flagellar movements were relatively quiescent, and that the amplitude of the flagellar movement, as well as the linear velocity of the sperm, decreased (Fig. 3.3b). More importantly, the addition of the UVJ extracts extended the sperm lifespan *in vitro*. In the presence of the UVJ extracts, sperm swam vigorously even after 48 h of incubation, whereas in the absence of the extracts, sperm usually died within 5 h (data not shown). These results indicate the possibility that unknown molecules responsible for sperm maintenance exist in the UVJ extracts. In the previous study, we also observed that the formation of secretory granules in the SST epithelial cells fluctuated during the



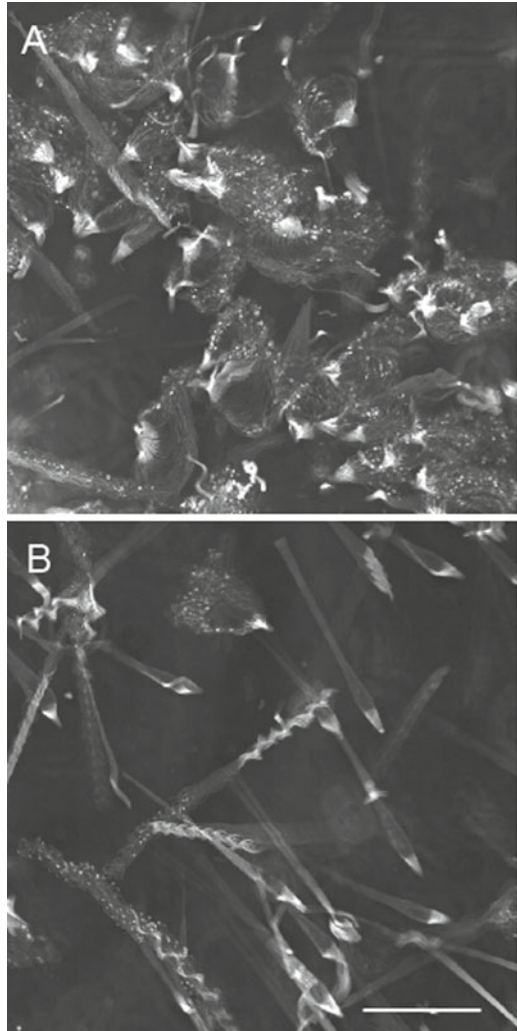
**Fig. 3.2** Ultrastructural observation of the resident sperm in the SST. The UVJ was isolated at 1 h after mating, and the tissue was embedded in paraffin wax. Thick sections were prepared, and the surface of the cross section was observed by scanning electron microscopy. *Arrow* indicates the bundle of resident sperm in the SST. A representative photograph from those obtained from three different birds is shown. *Bar*=5  $\mu$ m

ovulatory cycle, and progesterone treatment mimicking the phenomena takes place during the ovulatory cycle. In SST cells, there are well-developed tight junctions among the cells in the apical region, and the SST epithelial cells appear to secrete their contents into the lumen of the SST, where the resident sperm are located. Although we did not elucidate the nature of the secretory granules, it is very likely that the contents of the granules in the UVJ extracts affect sperm physiology (i.e., sperm filling, storage, and release) in the SST.

### 3.4 Conclusion

In this chapter, we reported that sperm maintenance in the SST, as well as sperm release from the same location, are events regulated during the ovulatory cycle. For instance, we demonstrated that progesterone stimulates the release of resident sperm from the SST in the Japanese quail with a contraction-like morphological change of the SST. This process may be supported by the lubricant effect of cuticle materials secreted from the ciliated cells of the UVJ, as well as unknown materials supplied from the SST epithelial cells, in events coincidentally triggered under progesterone control. In addition, we found secretory granules in SST epithelial cells, and the number of the secretory granules fluctuated during the ovulatory cycle, indicating that SST epithelial cells unknown materials into the lumen of the SST; these materials may affect sperm physiology (e.g., motility, respiration, and metabolism) (Ito et al. 2011). Although the nature of the molecules responsible for sperm maintenance for a long period of time remains to be clarified, we found extracts of UVJ possess the ability to reduce sperm motility and to extend sperm lifespan *in vitro*. Because sperm storage in

**Fig. 3.3** Effects of UVJ extracts on motility of ejaculated sperm incubated in the presence (**b**) or absence (**a**) of the UVJ extracts (300  $\mu\text{g}/\text{ml}$ ) at 39 °C for 10 min. The flagellar movements of the sperm were recorded by a high-speed camera (200 frames per second), and 10 images taken at every 1/20 s were overlaid. A representative photograph from those obtained from three different birds is shown. *Bar* = 100  $\mu\text{m}$



avian species occurs at high body temperature (i.e., 41 °C), elucidation of the mechanism for sperm storage may lead to the development of new strategies for sperm preservation at ambient temperatures.

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