Title: Sperm Storage in the Female Reproductive Tract in Birds

Author(s): Sasanami, Tomohiro; Matsuzaki, Mei; Mizushima, Shusei; Hiyama, Gen

Publication: Journal of Reproduction and Development. 59(4), p. 334-338

URL: http://hdl.handle.net/10297/9098

Copyright: © 2013 by the Society for Reproduction and Development
Sperm Storage in the Female Reproductive Tract in Birds

Tomohiro SASANAMI1), Mei MATSUZAKI1), Shusei MIZUSHIMA1) and Gen HIYAMA1)

1)Department of Applied Biological Chemistry, Faculty of Agriculture, Shizuoka University, Shizuoka 422-8529, Japan

Abstract. The ability to store sperm 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, 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 of sperm storage in the female reproductive tract has been poorly understood until recently. In avian species, the specialized simple tubular invaginations referred to as sperm storage tubules (SSTs) are found in the oviduct as a sperm storage organ. In this review, we summarize the current understanding of the mechanism of sperm uptake into the SSTs, maintenance within it, and controlled release of the sperm from the SSTs. Since sperm storage in avian species occurs at high body temperatures (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, and these could be used in a myriad of applications in the field of reproduction.

Key Words: Birds, Fertilization, Japanese quail, Progesterone, Sperm storage tubules, Utero-vaginal junction

In avian species, specialized simple tubular invaginations referred to as sperm storage tubules (SSTs) are found in the oviduct [10–13]. Because of the presence of this structure, once ejaculated sperm have entered the female reproductive tract, they can survive up to 2–15 weeks in domestic birds, including chickens, turkeys, quails, and ducks, depending on the species [14, 15] in contrast to the relatively short life span of mammalian spermatozoa (i.e., several days). SSTs are found in the lamina propria of mucosal folds in the uterus, where spermatozoa are transported to the infundibulum, although the primary storage site for sperm is the SSTs in the UVJ [13, 16]. Spermatozoa are transported to the infundibulum, which is the site of fertilization and also serves as a secondary sperm storage site [11, 17].

Bakst et al. [18] reported that the biological basis of sustained fertility in chicken and turkey hens is their capacity for sperm to reside in the SSTs of the UTJ, and the differences in the duration of fertility between domestic fowl (2 to 3 weeks) and turkeys (10 to 15 weeks) are, in part, related to their respective numbers of SSTs (the mean numbers of SSTs for chickens and turkeys are 4,893 and 30,566, respectively). Although extensive investigation concerning the function of the SSTs in birds has been done since their discovery in the 1960s using ultrastructural analysis [10, 19, 20], we currently know little about the specific mechanisms involved in sperm transport into the SSTs, maintenance within them, and controlled release of the sperm from them.

In this review, we summarize the current understanding of the mechanism of sperm storage in avian oviducts. In addition, we introduce our recent findings on the mechanism of sperm release from the SSTs in the Japanese quail (Coturnix japonica).

Sperm Uptake into the SSTs

Because of a thick and opaque oviductal wall in avian species, it is difficult to observe sperm movement in the oviduct directly. Therefore, we lack basic knowledge of how the sperm are transferred into the SSTs after insemination. After natural mating, the ejaculated sperm are deposited into the vagina; however, it is reported that more than
We currently do not know whether a chemotaxis event participates in the process of sperm uptake into the SSTs, but we observed that the filling rate of the SSTs after a single insemination in the quail was approximately 30–50% and that the sperm-filled SST were not uniformly distributed in the UVJ [25]. In addition, when the degree of sperm filling in the SSTs was categorized as being full, partially full, or empty, it differed considerably in each SST in the chicken [26]. These results indicated that unknown mechanisms that affect sperm uptake into the SSTs may be present in avian oviducts, and remain to be elucidated in future studies.

**Sperm Maintenance in the SSTs**

The period of fertility in birds is correlated with the population of sperm-filled SSTs in the UVJ. It is assumed that the SSTs supply nutrients to the sperm and remove any waste products of sperm metabolism [20]; however, the mechanisms by which the resident sperm are maintained in the SST for extended periods have not yet been elucidated. In the lumen of the SSTs, sperm appear as packed, parallel bundles with their heads directed toward the blind tubular end [19, 25]. In addition, it is reported that the sperm stored in the SSTs are typically immotile [27, 28] and thus thought to be metabolically quiescent as a result of lowered ATP consumption. This is a reasonable strategy because it also leads to reduced production of reactive oxygen species due to sperm respiration, and it may reduce damage to the resident sperm in the SSTs. Thus, the SSTs bring the stored sperm to a stop may be one of the most important factors in the maintenance of sperm in the SSTs. However, another idea has also been suggested in the chicken: sperm maintain their position against an outward fluid stream in the SSTs via the sperm motility generated by oxidation of exogenous fatty acids released from SST epithelial cells [14]. In this model, sperm efflux is thought to be the result of reduced sperm velocity due to a shortage of energy supplementation from sperm mitochondria. We observed the resident sperm in the UVJ mucosa of the Japanese quail immediately after isolation from birds at 1 h post-mating with non-fixed whole mount specimens. Although we cannot deny the possibility that unexpected changes happened to the sperm due to isolation, we were not able to find any movement of resident sperm in the SSTs.

As suggested by Van Krey et al. [29] and Froman and Engel [30], the resident sperm agglutinate head to head in the SSTs of the chicken. We also observed in the quail using light or electron microscopy that most of the sperm in the SSTs attach to each other in a bundle-like agglutination, and single sperm were seldom seen [25]. The tendency of sperm agglutination may be the basis for prolonged *in vivo* storage of spermatozoa because this style of sperm residency is common among domestic birds. In addition, several proteins including carbonic anhydrase [31], avidin [32], aquaporins [33] and alkaline phosphatase [34] have been suggested to have potential roles in sperm maintenance in the SSTs, although no direct implication in sperm storage has been demonstrated. Another important factor supporting sperm storage in the SSTs is defense from anti-sperm immune responses in the oviduct. This is because the immune system in the oviduct is well developed to protect it from infection by various microorganisms. This immune system may also affect the survivability of the sperm in the SSTs when the sperm are recognized as foreign bodies in the oviduct. Das et al.
[21] demonstrated that the resident sperm in the SSTs are protected from immune responses by SST structures and transforming growth factor β (TGFβ), the expression of which increased when the SSTs were filled with sperm. Since TGFβ and the receptors for TGFβ are also expressed in sperm, the enhanced expression of TGFβ and its receptors may protect sperm in the SSTs by suppressing anti-sperm immunoreactions. Thus, the elimination of anti-sperm immune responses by the TGFβ system is one of the factors responsible for sperm maintenance in the SSTs.

We also investigated the mechanism of sperm storage in the Japanese quail. It is reported that sperm at the uterotubal junction (UTJ) in the bovine oviduct bind to the surface of epithelial cells, and that this binding ensures the tethering of the sperm at the UTJ until the time of ovulation [4, 35]. In contrast to the situation in mammalian species, the resident sperm seem to be free from SST epithelial cells in the quail oviduct (Fig. 2). This finding led us to hypothesize that unknown materials in the lumen of the SSTs may affect sperm mobility. In order to confirm this hypothesis, we prepared UVJ extracts, and ejaculated sperm were incubated in the presence or absence of the 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, panel A). 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, panel B). More importantly, the addition of UVJ extracts extended the sperm’s lifespan in vitro. In the presence of UVJ extracts, sperm swam vigorously even after 48 h of incubation, whereas in the absence of the extracts, they usually died within 5 h (data not shown). These results indicate the possibility that unknown molecules responsible for sperm maintenance exist in UVJ extracts. In a previous study, we also observed that the formation of secretory granules in SST epithelial cells fluctuated during the ovulatory cycle [25]. In SST cells, there are well-developed tight junctions among the cells in the apical region, and SST epithelial cells appear to secrete their contents into the SST lumen, 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 UVJ extracts affect sperm maintenance in the SSTs.

**Sperm Release from the SSTs**

To achieve fertilization, the resident sperm must be released from the SSTs in order to migrate to the site of fertilization, which is infundibulum part of the oviduct. There are several reports indicating that sperm release from the SSTs is not regulated but occurs in response to the mechanical pressures of a passing ovum, as no contractile elements associated with the SSTs were found [20, 36]. In addition, Burke and Ogasawara [16], who recovered sperm from an inseminated hen oviduct, concluded that sperm release from the SSTs is a slow and continuous event that occurs constitutively during the ovulatory cycle. In contrast, Bobr et al. [10], who investigated the distribution of spermatozoa in the hen oviduct after insemination, reported that the resident spermatozoa were discharged from the SSTs close to the times of ovulations and/or ovipositions. In addition, Mero and Ogasawara reported that tubular enlargement of the SSTs is associated with sperm release in the chicken [36]. Moreover, Freedman et al. demonstrated the presence of neurons, small ganglia and F-actin in the UVJ of the turkey oviduct and suggested that an unknown neural factor may play a role in the functions related to sperm storage and release from the SSTs [37]. These observations indicated that the timing of the sperm release from the SSTs could be regulated hormonally. To examine whether sperm release from the SSTs is regulated during the ovulatory cycle, a female quail was mated 12 h after oviposition, and the SSTs in the UVJ at 2 or 13 h after mating (corresponding to a time 14 or 25 h after oviposition, respectively) were observed. The percentage of the SSTs filled with sperm at 14 h after oviposition was high (i.e., approximately 50–60%) and decreased significantly to approximately 40% at 25 h. Also, a bundle of sperm extruding into the lumen of the UVJ from the SSTs was frequently seen at 20 h after oviposition, while no such sperm were observed at 8, 14 or 25 h. To test whether hormonal stimulation causes sperm release from the SSTs, birds were injected with various steroid hormones, and the SST filling rate was calculated. As a result, the percentage of SSTs with sperm was only significantly decreased when the animals were treated with more than 0.8 μg/ml progesterone compared with that of control birds injected with a vehicle alone. Scanning electron microscopic observation revealed that the SSTs shrank due to the injection of progesterone, and a bundle of the sperm tail extruded from the SSTs was observed (Fig. 4). This morphological change showed that the SSTs squeezed out the resident sperm into the lumen of the oviduct. These results demonstrated that the release of sperm from the SSTs is a regulated event during the ovulatory cycle, and that progesterone acts as a sperm-releasing factor in birds [25]. If the resident sperm are released from the SSTs without any regulation, most of the sperm ascending the oviduct may be trapped by the descending egg. It is reasonable to suppose that sperm release from the SSTs is stimulated...
by progesterone because there is at least a 5-h grace period before the next ovulation and sperm released from the SSTs can reach the site of fertilization without hindrance from the descending egg. 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 SST epithelial cells, in events coincidently triggered under progesterone control. In addition, we observed secretory granules in SST epithelial cells, and the number of these secretory granules fluctuated during the ovulatory cycle, indicating that SSTs epithelial cell derived unknown materials secreted into the lumen of the SST may affect sperm physiology (e.g., motility, respiration, metabolism, etc.) [25]. Although the nature of the molecules responsible for long-term sperm maintenance remains to be clarified, our findings indicated that UVJ extracts possess the ability to reduce avian sperm motility and to extend sperm life span in vitro. Because sperm storage in avian species occurs at a 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, and these could be used for a myriad of applications in the field of reproduction.

**Acknowledgments**

This work was supported in part by financial support from a Grant-in-Aid for Scientific Research on Innovative Areas (24112710 to TS) and Grant-in-Aid for Scientific Research (B) (24380153 to TS).

**References**

1. Holt WV. Mechanism of sperm storage in the female reproductive tract: an interspecies comparison. Reprod Dom Anim 2011; 46: 68–74.
2. Holt WV, Lloyd RE. Sperm storage in the vertebrate female reproductive tract: How does it work so well? Theriogenology 2010; 73: 713–722. [Medline]
3. Birkhead TR, Møller AP. Sexual selection and the temporal separation of reproductive events: sperm storage data from reptile, birds and mammals. Biol J Linn Soc 1993; 50: 295–311.
4. Suarez SS. How do sperm get to the egg? Bioengineering expertise needed! Exp Mech
8. Froman D. Deduction of a model for sperm storage in the oviduct of the domestic fowl (Gallus domesticus). Biol Reprod 2003; 69: 248–253. [Medline]

9. Froman D. Sperm storage in the ovicervix of the chicken. PLoS Biol 2011; 9: e1001191. [Medline]

10. Grant P. Spermatogenesis and development in the chicken. Adv Anat Embryo 1957; 1: 1–118. [Medline]

11. Holm G, Sonstegard TS, Long EL, Birkhead TR, Madsen MF, Tassell CPV, et al. Ultrastructural and functional analysis of the ovarian cycles of birds. Reproduction 2006; 132: 129–138. [Medline]

12. Freedman SL, Akuffo V. Analysis of gene expression in sperm storage tubules of domestic fowl. Poult Sci 2007; 86: 753–760. [Medline]

13. Kupfer M, Bakst MR. Localisation of carbonic anhydrase in the sperm storing regions of the domestic hens oviduct. Acta Anat 1996; 156: 97–103. [Medline]

14. Holm G, Sonstegard TS, Long EL, Birkhead TR, Madsen MF, Tassell CPV, et al. Ultrastructural and functional analysis of the ovarian cycles of birds. Reproduction 2006; 132: 129–138. [Medline]

15. Mero FX, Griffith GB, Harris SC, et al. Localization of carbonic anhydrase in the sperm storing regions of the domestic hen oviduct. Acta Anat 1996; 156: 253–260. [Medline]

16. Long EL, Sonstegard TS, Long JA, Tassell CPV, Zuelke KA. Sperm selection by females. Reproduction 2004; 128: 1209–1211. [Medline]

17. Kupfer M, Bakst MR. Localization of aquaporins in the sperm storage tubules in the turkey ovicervix. Poult Sci 2004; 83: 1209–1212. [Medline]

18. Kupfer M, Bakst MR. Alkaline phosphatase reactivity in the vagina and utero-vaginal junction sperm storage tubules of turkeys in egg production: implications for sperm storage. Br Poult Sci 2007; 48: 513–518. [Medline]

19. Kupfer M, Bakst MR. Alkaline phosphatase reactivity in the vagina and utero-vaginal junction sperm storage tubules of turkeys in egg production: implications for sperm storage. Br Poult Sci 2007; 48: 513–518. [Medline]

20. Sonstegard TS, Long EL, Birkhead TR, Madsen MF, Tassell CPV, et al. Ultrastructural and functional analysis of the ovarian cycles of birds. Reproduction 2006; 132: 129–138. [Medline]

21. Kupfer M, Bakst MR. Localization of aquaporins in the sperm storage tubules in the turkey ovicervix. Poult Sci 2004; 83: 1209–1212. [Medline]

22. Kupfer M, Bakst MR. Alkaline phosphatase reactivity in the vagina and utero-vaginal junction sperm storage tubules of turkeys in egg production: implications for sperm storage. Br Poult Sci 2007; 48: 513–518. [Medline]

23. Hunter RHE. Sperm release from oviduct epithelial binding is controlled hormonally by peri-ovulatory graafian follicles. Mol Reprod Dev 2008; 75: 167–174. [Medline]

24. Mero FX, Ogasawara FY. Dimension of uterovaginal sperm-storage tubules of the chicken and their possible significance in sperm release. Poult Sci 1970; 49: 1304–1308. [Medline]

25. Friedman SL, Akuffo VG, Bakst MR. Evidence for the innervation of sperm storage tubules in the ovicervix of the turkey (Meleagris gallopavo). Reproduction 2001; 121: 809–814. [Medline]