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Localization of Oviductal Sperm-storage Tubules in the American Kestrel (*Falco sparverius*)

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Sperm-storage tubules (SST) are discrete tubular invaginations of the bird's oviduct epithelium located in the anterior end of the vaginal folds, a region generally referred to as the uterovaginal junction (UVJ). [We prefer to refer to the UVJ sperm-storage sites collectively as the SST (originally used by Mero and Ogasawara 1970) because SST accurately describes their function and structure.] Of the 27 recognized orders of birds, SST have been identified histologically only in selected species of Charadriiformes and Procellariformes (Hatch 1983), Galliformes (Fujii and Tamura 1963), Anseriformes (Pal 1977), and Passeriformes (Bray et al. 1975). Whether SST are structures common to all birds, as suggested by Gilbert (1979) and Hatch (1983), remains to be investigated.

The presence of SST has not been demonstrated histologically in the Falconiformes. The high frequency of copulations in the course of laying one clutch of eggs prompted Corten (1973) to suggest that SST do not exist in the Northern Goshawk (*Accipiter gentilis*). Bird and Buckland (1976) observed the mean duration of fertility following artificial insemination of the American Kestrel (*Falco sparverius*) to be 8.1 days (range = 4–12 days). They suggested that SST were present in the oviduct. We present evidence that SST exist at the UVJ of the American Kestrel. In addition, a technique for the precise localization and isolation of oviductal mucosa containing SST is described.

Localization and isolation of SST.—Three females maintained as previously described (Bird 1982) were killed within 48 h of artificial insemination. The oviducts were removed, immersed *in toto* into neutral buffered formalin (NBF), and stored (6-8 weeks) at room temperature until processed. Whole oviducts were transferred to Mirskey's fixative (National Diagnostics) for about 24 h before gross dissection. For the precise localization and visualization of SST, the connective tissue that binds the vagina in a tightly coiled structure between the uterus and the cloaca was stripped. By carefully removing thin strips of connective tissue with fine forceps and scissors, the vagina was exposed and straightened. The vagina and uterus were cut along the long axis of the oviduct and then pinned to a dissection board. The exposed mucosa was kept moist with phosphate-buffered saline (PBS). Individual mucosal folds were then removed with iris scissors, immersed in a plastic dish containing PBS, and examined under a Zeiss SR Stereophotomicroscope using transillumination (the light beam is parallel to the base of the microscope and directed to the isolated fold). With unfixed chicken or turkey specimens, long pieces of tissue (3–4 cm) can be isolated (M. Bakst unpubl. data). In fresh, unfixed tissue the SST are easily visualized and can be manipulated to facilitate making squash preparations. However, fixation renders the mucosa surrounding the SST more opaque and considerably less pliable. Pieces of NBF-fixed American Kestrel UVJ mucosa less than 1 mm long were examined as above to visualize individual SST. Once the precise location of the SST was determined, the oviductal segment containing the SST was excised and processed for light microscopy. Paraffin sections (5–6 μm thick) were stained with periodic-acid Schiff (PAS) with and without a hematoxylin (H) counterstain.

Morphology of the UVJ and SST.—Macroscopic inspection of the American Kestrel uterus and vagina revealed longitudinally oriented folds that are relatively wide at the uterus, narrow abruptly at the UVJ, and then continue through the vagina (Fig. 1). Light microscopy revealed an oviductal luminal surface epithelium that consisted of alternating columnar ciliated and nonciliated secretory cells, the latter stained considerably more intensely with PAS/H than the former. Tubular glands (Fig. 2) underlie the surface epithelium in the uterus, and probably contribute to its more voluminous folds (compared with the vagina). The abrupt termination of the uterine folds observed macroscopically (Fig. 1) is seen by light microscopy as an abrupt cessation of the tubular glands (Fig. 2). No transitional forms of uterine tubular glands or SST were observed. Sperm-storage tubules were located in a band of UVJ mucosa about 3–4 mm wide, indicated by the probe in Fig. 1.

The surface epithelium at the UVJ is composed of ciliated and nonciliated secretory cells, the latter apparently stained more intensely with PAS/H (Figs. 3–5). Serial sections of this region indicate that some SST may be coiled, while others are relatively straight tubular invaginations of the surface epithelium. The epithelium around the SST orifice, and for a short distance into the SST, appears to be a continuation of the UVJ surface epithelium (Fig. 5). There is an abrupt transition between this epithelium and the

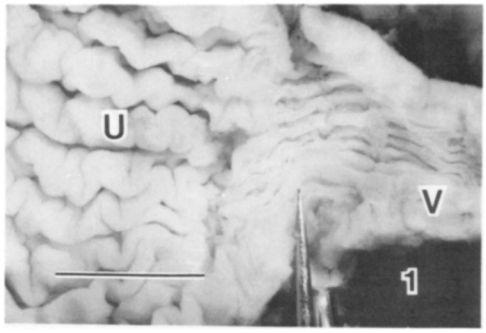

Fig. 1. The uterine (U), uterovaginal junction (UVJ; probe), and vaginal (V) mucosae of *F. sparverius*. The probe points to the location of the band (3–4 mm wide) of UVJ mucosa containing sperm-storage tubules. Bar = 2 mm.

epithelium lining the SST. The latter consists of nonciliated columnar cells that contain variable amounts of a fine, granular PAS-positive material, possibly glycogen, in the supranuclear cytoplasm (Figs. 3–5). No sperm were observed.

Glandular grooves are located deep in the secondary folds of the distal infundibulum (Fig. 6). The epithelium that forms the glandular grooves contains low columnar, nonciliated, PAS-negative epithelial cells that form a slightly concave pocket. These structures were shown to store sperm in the chicken (Van Drimmelen 1946, Fujii and Tamura 1963) and turkey (Bakst 1981) and generally are regarded as secondary to the UVJ-SST with respect to sperm storage. Whether they have the capacity to store sperm in the American Kestrel is not known.

Significance of oviductal sperm storage.—The breeding behavior of the American Kestrel involves multiple copulations before laying the first egg (Balgooyen 1976). The role of the SST in the reproductive process of the American Kestrel is not clear. The necessity of oviductal sperm storage in birds in general was addressed by Hatch (1983). Citing Lake (1975), Hatch (1983) noted that clutch size, mating system, and prevalence of renesting were factors that "... may determine the selective value of oviductal sperm storage." He added a fourth factor, which he termed "delayed fertilization." Briefly, in this situation one individual (usually the female) leaves its partner after copulation to forage but returns to the nest site before the onset of laying.

We suggest that the function of oviductal sperm storage must be considered in the context of the fate of sperm within the oviduct. The majority of sperm artificially inseminated are expelled from the vagina within 1 h of insemination (Howarth 1971), and the UVJ acts as a barrier to adovarian sperm transport (Allen and Grigg 1957), permitting only viable sperm to enter the SST (Lake 1975). Also, sperm release from the SST is either continuous or episodic during the daily ovulatory cycle (Bakst 1981), but is reduced significantly when the hen is anovulatory (Bushman et al. 1985). Furthermore, an oviductal ovum depletes significantly the number of sperm within the oviductal lumen (Bakst 1981). Finally, the time span in which the ovulated ovum is accessible to the sperm at the site of fertilization appears to be quite short,

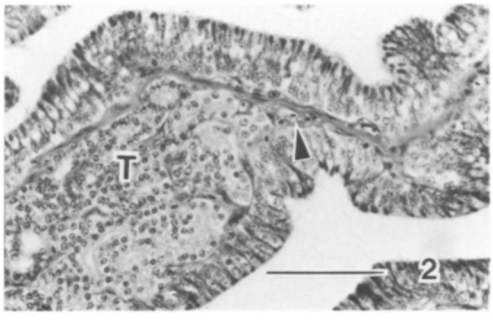

Fig. 2. The abrupt termination (arrow) of the uterine tubular glands (T) at the uterovaginal junction. This region corresponds to the region indicated by the probe in Fig. 1. Bar = 50 μm.

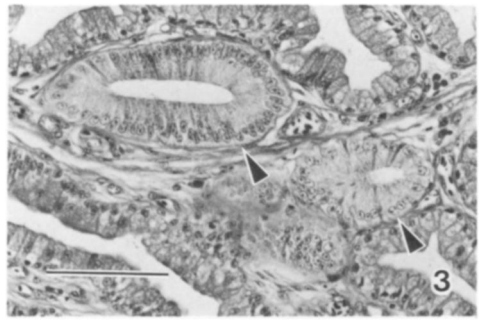

Fig. 3. SST (arrows) subjacent to the PAS-positive surface epithelium. (PAS/H.) Bar = 50 μm.

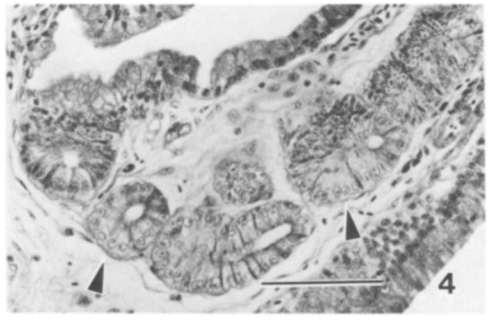

Fig. 4. A section through the longitudinal axis of two SST (arrows) to illustrate the coiled form of the SST. Bar = 50 μm.

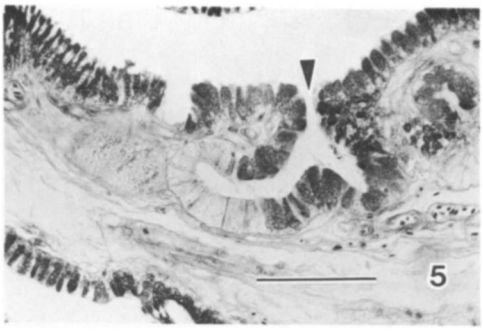

Fig. 5. The opening to the SST (arrow). (PAS without counterstain.) Bar = 50 μm.

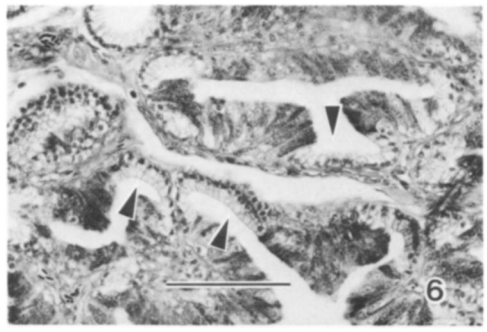

Fig. 6. Slightly concave, PAS-negative, light-staining, glandular grooves (arrows) are deep in the secondary folds of the distal infundibulum. Bar = 50 μm.

because the deposition of oviductal secretory material around the ovum, which begins at the distal infundibulum, renders the ovum impenetrable to sperm (Bakst and Howarth 1977). We suggest that the SST are necessary to ensure that a population of sperm adequate to maintain sustained fertility is present at the site of fertilization following each ovulation. The mechanism that initiates and maintains the continuous release of sperm from the SST at the onset of egg production is not known.

The observation that only viable sperm enter the SST (Lake 1975) suggests another role for the SST. The number of sperm in a manually collected semen sample from the American Kestrel was low (416,000 sperm in 12 μl of ejaculate) in comparison with other birds (Bird and Laguë 1977, Gee pers. comm.). Perhaps the high number of copulations before the onset of laying is necessary for the filling, or partial filling, of American Kestrel SST with viable sperm. This population of "selected" sperm would then be released to populate the site of fertilization at the anterior oviduct under the appropriate stimuli at the onset of and for the duration of egg production. Evidence presented elsewhere (Bird and Buckland 1976) suggests that the American Kestrel can store sperm for up to 11 days following artificial insemination.

Glandular grooves located in the deep mucosal infoldings at the distal infundibulum have been shown to store sperm in the chicken (Van Drimmelen 1946, Fujii and Tamura 1963) and turkey (Bakst 1981). Whether they have a similar capacity in the American Kestrel remains unknown. Two possible reasons for the absence of sperm from the histological preparations of the UVJ are that (1) only about 51% of the females subjected to artificial insemination lay fertile eggs (Bird et al. 1976), which would suggest that sperm may not reach the SST in half the birds inseminated, and (2) the population of American Kestrels used in this study was approaching the end of the breeding season and consequently the semen quality and the efficacy of sperm transport and sperm-storage mechanisms may have waned to such an extent as to preclude sperm acceptance into the SST.

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Literature Cited

Allen, T. E., & G. W. Grigg. 1957. Sperm transport in the fowl. Australian J. Agr. Res. 8: 788–799.

Bakst, M. R. 1981. Sperm recovery from the oviducts of turkeys at known intervals after insemination and oviposition. J. Reprod. Fert. 62: 159–164.

———, & B. Howarth. 1977. Hydrolysis of the hen's perivitelline layer by cock sperm *in vitro*. Biol. Reprod. 17: 370–379.

Balgooyen, T. G. 1976. Behavior and ecology of the American Kestrel (*Falco sparverius* L.) in the Sierra Nevada of California. Univ. California Publ. Zool. 103: 1–83.

Bird, D. M. 1982. The American Kestrel as a laboratory research animal. Nature 299: 300–301.

———, & R. Buckland. 1976. The onset and duration of fertility in the American Kestrel. Can. J. Zool. 54: 1595–1597.

———, & P. C. Laguë. 1977. Semen production in the American Kestrel. Can. J. Zool. 55: 1351–1358.

———, ———, & R. B. Buckland. 1976. Artificial insemination vs. natural mating in captive American Kestrels. Can. J. Zool. 54: 1183–1191.

Bray, O. E., J. J. Kennelly, & J. L. Guarino. 1975. Fertility in eggs produced on territories of vasectomized Red-winged Blackbirds. Wilson Bull. 87: 187–195.

Bushman, A. F., H. P. van Krey, D. M. Denbow, & P. B. Siegel. 1985. Effect of the ovulatory cycle on oviductal sperm storage in the domestic fowl. Theriogenology 23: 474–479.

Corten, P. J. M. 1973. Through method and mach-

ination. Captive Breeding of Diurnal Birds of Prey 1: 3–5.

FUJII, S., & T. TAMURA. 1963. Location of sperms in the oviducts of the domestic fowl with special reference to storage of sperms in the vaginal gland. J. Fac. Fish. Anim. Husbandry, Hiroshima Univ. 5: 145–163.

GILBERT, A. B. 1979. Female genital organs. Pp. 237–360 *in* Form and function in birds, vol. 1 (A. S. King and J. McLelland, Eds.). New York, Academic Press.

HATCH, S. A. 1983. Mechanism and ecological significance of sperm storage in the Northern Fulmar with reference to its occurrence in other birds. Auk 100: 593–600.

HOWARTH, B. 1971. Transport of spermatozoa in the reproductive tract of turkey hens. Poultry Sci. 50: 84–89.

LAKE, P. 1975. Gamete production and the fertile period with particular reference to domesticated birds. Symp. Zool. Soc. London 35: 225–244.

MERO, K. N., & F. X. OGASAWARA. 1970. Dimensions of uterovaginal sperm-storage tubules of the chicken and their possible significance in sperm release. Poultry Sci. 49: 1304–1308.

PAL, D. 1977. Histochemistry of the utero-vaginal junction with special reference to the sperm-host glands in the oviduct of the domestic duck. Folia Histochem. Cytochem. 15: 235–242.

VAN DRIMMELEN, G. C. 1946. "Spermnests" in the oviduct of the domestic hen. J. S. African Vet. Med. Assoc. 17: 43–52.

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