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Sperm-storage tubules in the vagina of the ostrich (*Struthio camelus*)

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ABSTRACT

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Sperm-storage tubules have been described in a number of species of birds. The presence of these tubules in the Rhea has been mentioned, but no description of these structures in ratites is available. The purpose of this study was to determine the presence and morphology of sperm-storage tubules in the vagina of the ostrich. The study was performed with the use of conventional light- and electron-microscopic techniques. Sperm-storage tubules were located in a 200-mm-wide band of the vagina adjacent to the utero-vaginal junction. The tubules were mostly branched and slightly coiled and lined by columnar epithelial cells. The cells contained a basal nucleus and displayed extensive apical junctional complexes. TEM revealed sperm in all the tubules examined.

Keywords: Sperm-storage tubules, vagina, ostrich, *Struthio camelus*

INTRODUCTION

In the animal kingdom various reproductive strategies are followed. In birds, one such strategy is the ability of the female to store sperm in specialized tubules, *Tubuli spermatici*, situated at the utero-vaginal junction of the oviduct. The sperm-storage tubules differentiate from the luminal epithelium of the vagina before the onset of laying (Bakst 1987). Their size and form differ between and within species (Shugart 1988) and can be straight, coiled, simple or branched. The number of sperm-storage tubules in various species has been estimated by Birkhead & Hunter (1990) and Birkhead & Møller (1992).

Sperm storage obviates the necessity to synchronize copulation and ovulation while eggs are being laid. Birkhead, Atkin & Møller (1987) showed that, in the majority of species, the frequency of copulation drops dramatically after the start of egg laying and in many cases ceases several days before the end of the female's fertile period. Birkhead (1992) showed that female Bengalese finches are capable of producing fertile eggs for up to 16 d following the last copulation. In some species of birds the period can even be longer, varying from 6–45 d (Birkhead & Møller 1992). Whether female birds of all species have the capacity or need to store sperm is not known (Astheimer 1985).

In Bengalese finches, on average, only about 1% of sperm transferred during copulation is stored, thus allowing a population of sperm to be "selected" from the ejaculate (Birkhead 1992). A similar "selection" of sperm was reported by Bakst & Bird (1987) in the American kestrel. Furthermore, Steele & Wishart (1992) showed that a barrier to foreign spermatozoa exists in the vagina of the fowl, thereby allowing only

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fowl spermatozoa to pass proximally in the oviduct. Muwazi, Baranga, Kayanja & Schliemann (1982) studied the histology of the oviduct obtained from three ostriches just before oviposition. The authors state that there are no glands in the vagina of the ostrich and they do not describe any structure resembling sperm-storage tubules.

Present developments in the ostrich industry indicate that artificial insemination may be a means by which the cost of breeding could be substantially reduced. To optimize artificial insemination, a thorough knowledge of reproduction, including sperm storage, is essential. The present study was therefore undertaken to determine whether sperm-storage tubules are present in the ostrich and, if so, to describe their structure and location.

MATERIALS AND METHODS

The oviducts of two ostrich females became available for study during the 1994 breeding season from commercial ostrich farms in the Oudtshoorn district. Both females suffered broken legs that could not be repaired and the birds had to be slaughtered. At the time of slaughter the entire oviducts (from the ovary to the cloaca) were removed, opened longitudinally and immersion-fixed in 10% formalin. The luminal surface of the oviduct presented longitudinal folds, 5–20 mm high, over the entire length of the uterus and vagina. After fixation, entire folds from the uterus and vagina were removed. Each fold was subsequently spread out flat and cut into 10-mm-long segments. For light microscopy, each segment was dehydrated through graded ethanols and embedded in Polaron Embedding Medium (JB4). After embedding, the blocks were trimmed and 4-µm-thick sections were cut on an Anglia Scientific rotary microtome. Each section was routinely stained with haematoxylin, counter-stained with eosin and examined under a light microscope. Photographs were taken of relevant areas.

Although the specimens were not collected specifically for electron microscopy, it was decided to prepare the material for ultrastructural investigation. A number of utero-vaginal folds were divided into segments as for light microscopy, and small blocks of tissue were trimmed from each region. The tissue blocks were rinsed twice in Millonig's phosphate buffer and then immersion-fixed overnight at 4°C in 4% glutaraldehyde in the same buffer. The blocks were subsequently rinsed in Millonig's phosphate buffer, post-fixed for 1 h at room temperature in similarly buffered 1% osmium tetroxide and given two final buffer washes. The samples were dehydrated through a graded ethanol series (25%, 50%, 75%, 96%, 100% x 2–10 min per step), cleared in propylene oxide and embedded in EMbed 812 epoxy resin. Semi-thin sections were prepared and stained with toluidine blue for light-microscopical examination. Thin sections were cut with a diamond knife on a Reichert OmU4 ultramicrotome, stained with uranyl acetate (Watson 1958) and lead citrate (Reynolds 1963), and examined with a Philips 301 or CM 10 transmission electron microscope operated at 80 kV.

RESULTS

On macroscopical examination of the fixed oviduct, a distinct difference between the mucosa of the uterus and that of the vagina was evident. The mucosa of the uterus was appreciably darker in appearance than that of the vagina and the transition from one to the other was abrupt and well demarcated (Fig. 1). The mucosa of both the uterus and vagina contained approximately 80 longitudinal folds that were 5–20 mm in height. Each of the mucosal folds had a scalloped or wavy appearance (Fig. 1).

Light microscopy

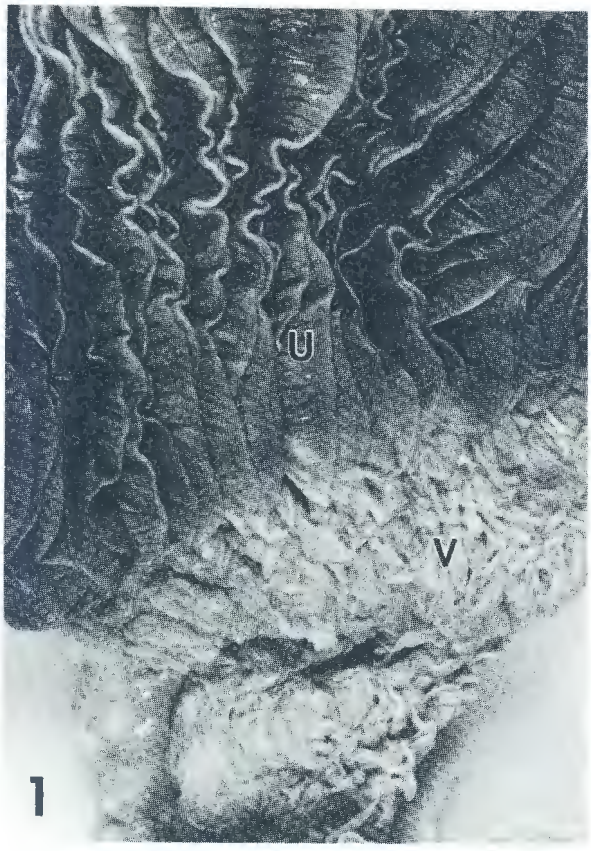
Light microscopically, the mucosal folds displayed numerous secondary (Fig. 3) and occasional tertiary folds. Each fold consisted of a lamina epithelialis resting on a basal lamina and supported by a lamina propria (Fig. 3–7). The epithelium of the uterus as well as that of the vagina consisted of ciliated and non-ciliated cells. The cytoplasm of both the cell types

FIG. 1 A macroscopic view of the utero-vaginal junction. Note the darker appearance of the uterine mucosa (U) compared to that of the vagina (V). The transition between the two regions is abrupt. The mucosal folds of the uterus are prominent and present a wavy appearance, whereas the vaginal folds appear less obvious owing to their more flimsy nature

FIG. 2 A low-power micrograph of the utero-vaginal junction showing the gland-rich mucosa of the uterus (U) and the sudden transition to the glandless mucosa of the vagina (V). A few sperm-storage tubules are present in the lamina propria of the vagina (arrowheads). LM. Toluidine blue x120

FIG. 3 A longitudinal section through a primary vaginal fold. Numerous secondary folds (arrows) are obvious and sperm-storage tubules (arrowheads) are seen to originate from the base of the folds. LM. H & E. x120

FIG. 4 A coiled sperm-storage tubule (arrow) situated in the lamina propria (LP) at the base of a vaginal fold. A second storage tubule is also obvious. Note that the nuclei of the epithelium of the storage tubules appear to be a continuation of the basal layer of nuclei of the ciliated vaginal epithelium (arrowheads). LM. H & E. x370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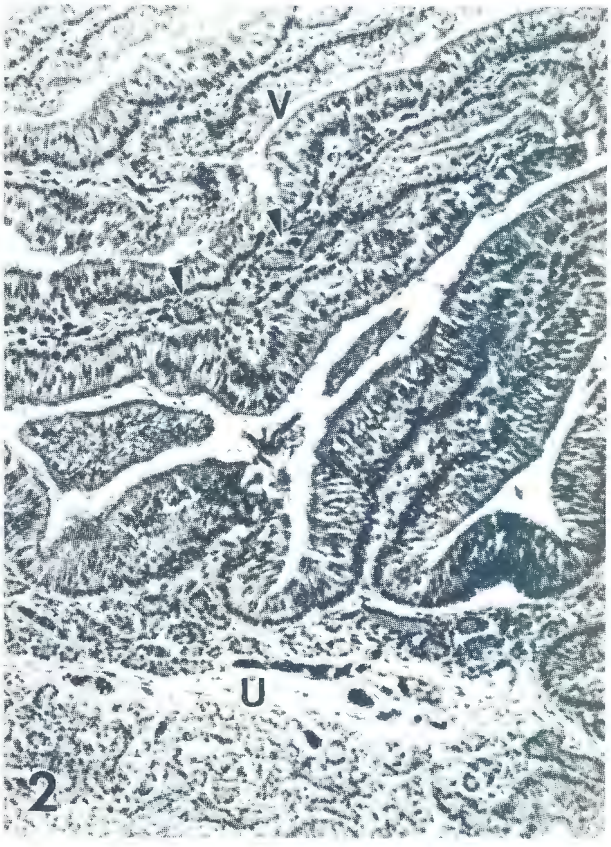
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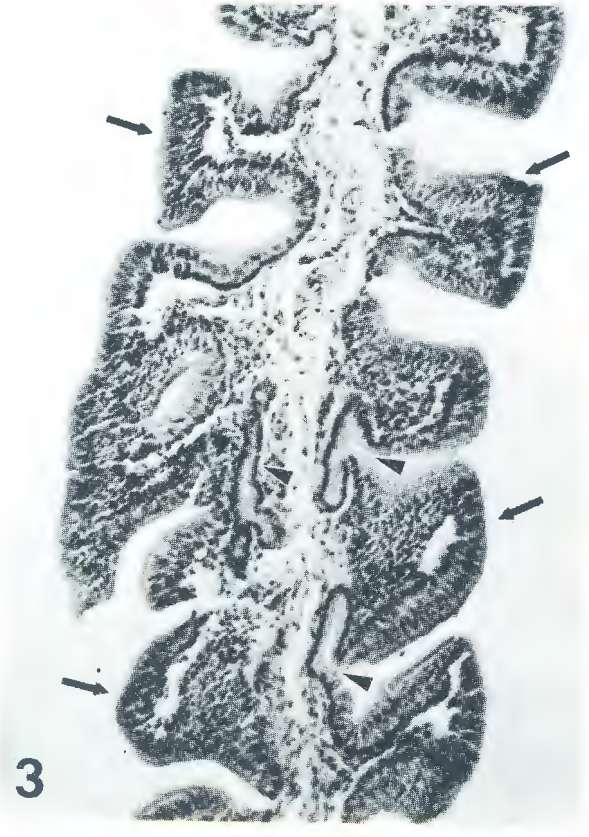
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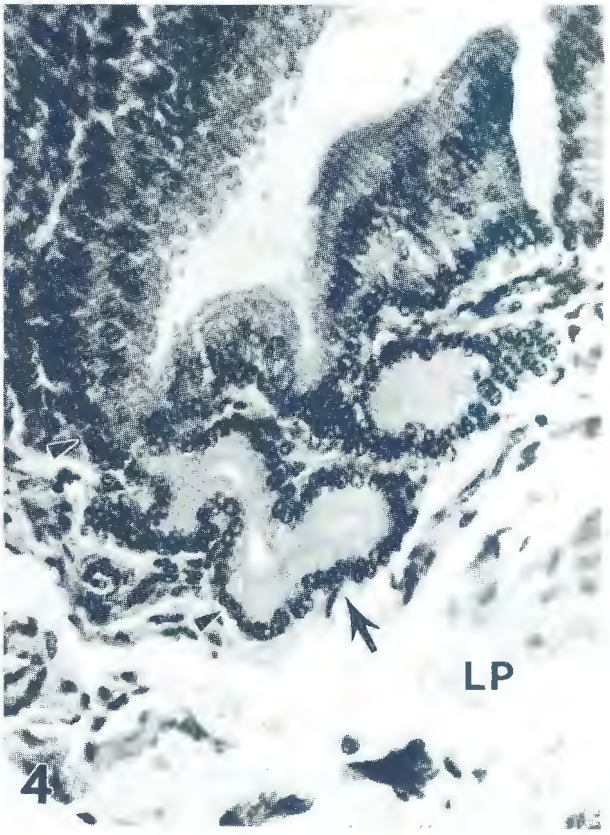
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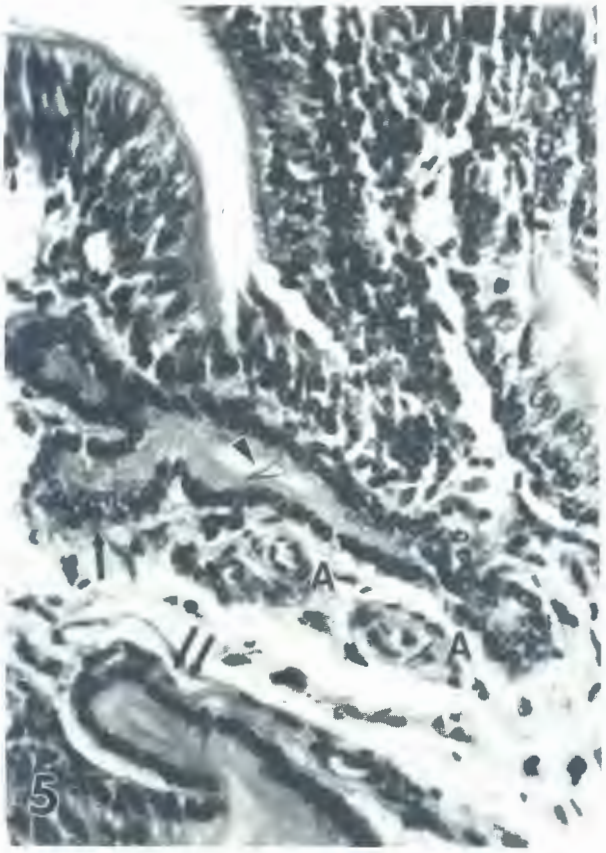
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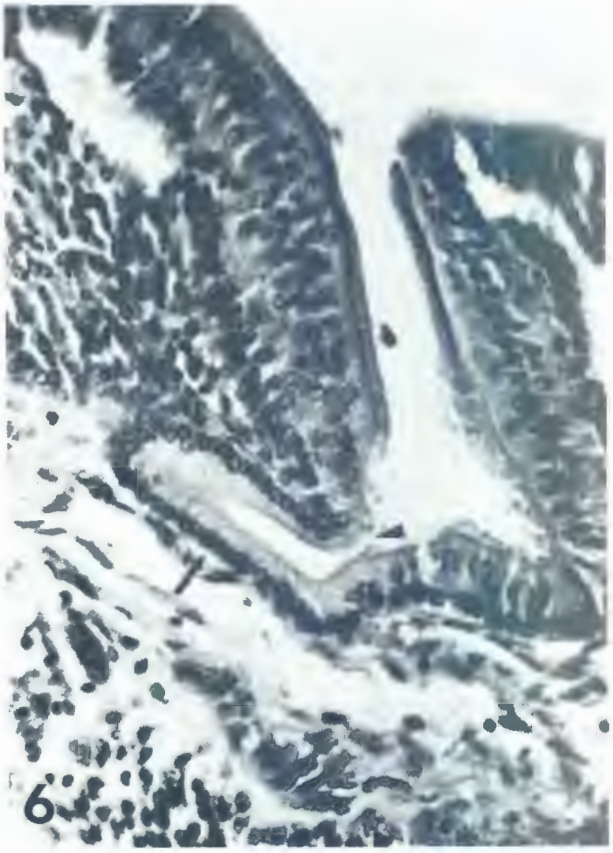
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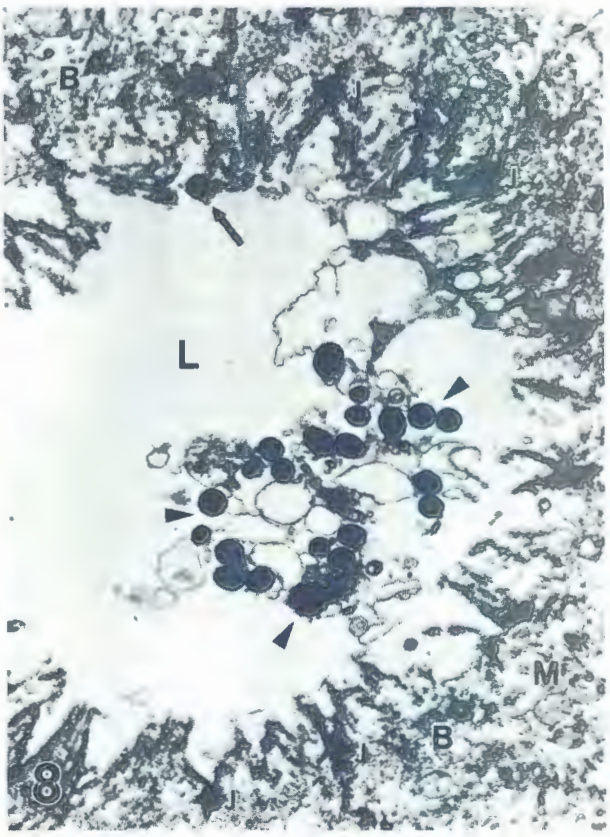
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was non-homogenous in appearance. Toluidine blue-stained sections revealed pale- and dark-staining cells (Fig. 7). The dark-staining cells appeared to be of the non-ciliated type and were relatively few in number. The nuclei of the epithelial cells were either apical, central or basal in position, giving the mucosa a stratified appearance (Fig. 6 and 7). The apical and central nuclei were oval to elongated and pale staining while the basal ones were round to oval and darker staining (Fig. 7). In the uterus large numbers of uterine glands were observed in the lamina propria, while the vagina did not display any evidence of glandular tissue (Fig. 2). However, many sperm-storage tubules (*Tubuli spermatici*) (Fig. 3–6), some containing spermatozoa (Fig. 5 and 8), were observed in a region of the vagina extending from the utero-vaginal junction over a distance of up to 200 mm. These tubules extended into the lamina propria from the bases of the secondary or tertiary mucosal folds and were mostly branched (Fig. 5) and slightly coiled (tortuous) (Fig. 4). Each tubule was lined by a columnar epithelium resting on a basal lamina (Fig. 4–7) and was situated in close proximity to blood vessels (Fig. 5). The cytoplasm of the tubular-lining cells was homogenous and contained a round, proximally situated nucleus with one or two prominent nucleoli (Fig. 4–7). The distal (luminal) ends of the cells presented a scalloped appearance. In toluidine blue-stained sections, distinct cell membranes with extensive apical junctional complexes and a prominent basal lamina were observed (Fig. 7). The transition from the epithelial lining of the vagina to the epithelial lining of sperm-storage tubules was very distinct and abrupt (Fig. 3, 4 and 6). Ciliated epithelial cells were not observed in the tubules.

Not all the tubules examined light microscopically contained sperm, and only the heads of the spermatozoa were readily observed. Patches of ill-defined grey material were sometimes visible within the lumen and presumably represented sectioned sperm tails. The sperm heads were distally oriented and bunched together in the distal part of the tubule.

Electron microscopy

The ultrastructural preservation of the tissue was poor owing to the inadequate and inappropriate fixation of the material for electron microscopy. The simple columnar cells forming the sperm-storage tubules displayed a large, pale, apical cytoplasm devoid of any recognizable cell organelles, except for occasional ill-defined mitochondria. However, extensive apical junctional complexes could be identified and appeared to be composed of tight junctions and desmosomes. A structure resembling a basal body was present at the apex of each cell (Fig. 8). The luminal surface of the cells was folded and the round, pale nucleus was situated near the base of the cells.

Sperm were identified in all the tubules examined and varied in number from 3–60. All the sperm were orientated along the length of the storage tubules, generally in a bundle in the centre of the lumen (Fig. 8). Some individual sperm were, however, more intimately associated with the walls of the tubules. Transverse views of any given tubule generally revealed sperm sectioned at a similar region, e.g. the nucleus, midpiece or principal-piece, indicating that all the sperm within a tubule adopted a similar lengthwise orientation (Fig. 8). However, some tubules revealed sperm sectioned at different levels, although in such instances the majority of the cells were similarly oriented. Although the sperm cells also revealed signs of inadequate fixation (swollen plasmalemma, poor mitochondrial detail, etc.) they displayed the general characteristics of mature ostrich sperm (Soley 1989; Baccetti, Burrini & Falchetti 1991; Soley 1993, 1994).

DISCUSSION

Comparatively few avian species have been investigated for the presence of sperm-storage tubules (Birkhead & Hunter 1990; Birkhead & Møller, 1992; Shugart 1988). No published work is available on the

FIG. 5 A branched sperm-storage tubule (arrow). Note the sperm-head profiles (arrowhead) within the lumen of the tubule and the proximity of two arterioles (A) to the tubule. An unbranched tubule (double arrow) is also shown on the micrograph. LM. H & E. x370

FIG. 6 An apparently unbranched sperm-storage tubule emanating from the base of a vaginal fold (arrow). The ciliated cells of the vaginal epithelium stop abruptly at the opening of the SST (arrowhead). Note the stratified appearance of the vaginal epithelium and the single layer of columnar cells lining the SST. The nuclei of the latter are continuous with the basal nuclei of the vaginal epithelium. LM. H & E. x370

FIG. 7 A transverse section of a sperm-storage tubule (SST) emerging from the base of a vaginal fold. The plane of section does not pass through the interconnecting lumen. Note the apical junctional complexes (arrowheads) surrounding the lumen of the SST and the well-developed basal lamina (arrow). The surface of the vaginal fold displays tufts of cilia (C) and the stratified epithelium is composed of dark- and light-staining cells. LM. Toluidine blue. x925

FIG. 8 A cross-section through the lumen (L) of a sperm-storage tubule revealing a group of sperm (arrowheads) sectioned at the level of the head. The sperm are centrally positioned within the lumen, while a single cell (arrow) is more intimately associated with the epithelial lining of the tubule. Although the ultrastructural preservation of the cells is poor, basal body-like structures (B), mitochondria (M) and junctional complexes (J) can be identified. TEM. x8850

existence of these structures in ratites, except for a reference to their presence in the rhea (Bakst 1987). The present study revealed the existence of sperm-storage tubules in the ostrich. In all species previously studied by various authors, sperm-storage tubules were found in a specialized region of the oviduct, 2–5 mm wide, referred to as the utero-vaginal junction (Bakst & Bird 1987; Birkhead & Hunter 1990). In the ostrich the utero-vaginal junction is macroscopically clearly demarcated. Sperm-storage tubules are found in an extensive region, 200 mm wide, on the vaginal side of the utero-vaginal junction. Therefore it is inappropriate, in the ostrich, to refer to the area containing sperm-storage tubules as the utero-vaginal junction. The extent of this region in the proximal vagina of the ostrich is possibly related to the physical size of the oviduct. The failure of Muwazi *et al.* (1982) to find glandular tissue (tubules) in the vagina of the ostrich is probably due to the fact that they obtained their samples only from the terminal vagina.

In the ostrich the mucosa of the vagina formed extensive primary, secondary and tertiary folds. This is in contrast to the findings of Muwazi *et al.* (1982) who reported only primary folds in the vagina, with little tendency to form secondary folds. Sperm-storage tubules differentiate from the mucosa on the sides and bases of the mucosal folds and extend into the lamina propria (Bakst 1987; Birkhead & Hunter 1990). There is an abrupt transition from the epithelial-surface lining of the vagina to the lining of the sperm-storage tubules in some species, whereas in others it is difficult to distinguish the tubules from the surrounding mucosa (Bakst 1987; Bakst & Bird 1987; Birkhead & Hunter 1990; Briskie & Birkhead 1993). In the present study, the transition from the vaginal epithelium to the tubular epithelium was found to be abrupt and clearly distinguishable. The tubules can be single or branched and straight, coiled, tortuous or bud-like (Bakst & Bird 1987; Shugart 1988; Birkhead & Hunter 1990; Birkhead, Pellatt & Hunter 1990; Bakst 1992; Bakst 1993). The branched tubules divide close to their origins (Bakst 1992). In the ostrich, the tubules branched close to their origins and were slightly tortuous.

The utero-vaginal junction of the oviduct of birds is lined by a pseudostratified columnar epithelium composed of predominantly ciliated cells and interspersed non-ciliated cells. (Bakst 1987; Bakst & Bird 1987; Bakst 1992). The nuclei of the ciliated cells are situated in the apical half of the cells, while the nuclei of the non-ciliated cells are located in the basal half of the cells (Bakst 1987). Muwazi *et al.* (1982) reported that the vaginal epithelium of the ostrich was composed of ciliated and many non-ciliated pseudostratified columnar cells with two layers of nuclei. In the present study, the vaginal epithelium of the region examined was found to consist mainly of ciliated cells with few non-ciliated cells situated between them. The elongated or oval nuclei of the ciliated cells were situated centrally or apically. The apical cytoplasm of these cells was wide, frequently creating the impression that the luminal surface was composed only of ciliated cells. The dark, round to oval nuclei of the non-ciliated cells were situated close to the basal lamina. The apical cytoplasm of these cells appeared to be extremely attenuated and was difficult to visualize between the ciliated cells in H & E preparations.

The sperm-storage tubules of birds are lined by a simple columnar epithelium with a basal nucleus (Bakst 1987; Bakst & Bird 1987; Shugart 1988). Our results confirmed this basic observation, but in addition revealed the presence of extensive apical junctional complexes. The morphology of the sperm-storage tubules is similar to that of the ducts of the uterine glands (Hatch 1983). However, the former are always fewer in number and characteristically display sperm in the lumen after insemination. Some authors have observed that when conventional light-microscopic techniques are used, not all sperm-storage tubules display sperm. This was also the case in the present study. McIntyre & Christensen (1983) estimated for, example, that in histological sections only 72% of the sperm-storage tubules contained sperm. With differential interference contrast and fluorescence microscopy of fresh, unfixed oviductal mucosa, it is possible to identify sperm in 90–95% of the tubules (Bakst 1993). In the present study, all the tubules examined by TEM, displayed sperm. In the turkey, luminal spermatozoa are distributed primarily in the distal third of the sperm-storage tubules and nearly always form tight bundles at the base of the tubule (Bakst 1992). A similar situation was also obvious in the ostrich. The observation that not all sperm-storage tubules examined light microscopically contained stored sperm, is possibly due to the thinness of the sperm tails, particularly when viewed in transverse section.

This study revealed the existence of sperm-storage tubules in the vagina of the ostrich. Their location at the utero-vaginal junction was similar to that of other birds, although they were distributed over a wider area.

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