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Alkaline phosphatase reactivity in the vagina and uterovaginal junction sperm-storage tubules of turkeys in egg production: implications for sperm storage

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Abstract 1. Currently there remains contradictory information on the localisation and possible role of alkaline phosphatase (AP) in the chicken and Japanese quail oviducts.
2. Using turkeys with a hard-shelled egg in their uteri, vaginal and uterovaginal junction mucosae were stretched and fixed as whole mounts prior to the histochemical localisation of AP activity.
3. Scattered AP reactive cells were observed in the vaginal and uterovaginal junction surface epithelia and intense AP reactivity of the sperm-storage tubule (SST) epithelium, localised to its apical border.
4. We suggest that such AP reactivity in hens in egg production may reflect cell differentiation and proliferation in the vagina and SST and possibly a mechanism for the transfer of lipid from the SST epithelia to resident sperm.

INTRODUCTION

The alkaline phosphatases (AP) are a class of cell-surface zinc metallo-enzymes that hydrolyse phosphate ester groups at an alkaline pH *in vitro*. Although highly conserved through species from bacteria to mammals, the precise roles of AP *in vivo* have not been defined. In chickens, AP activity has been found in a variety of tissues and cells including, but not limited to, the following: the reticular cells of bone marrow (Yoshida and Yumoto, 1987); intestine (Sharma *et al.*, 2000); blood plasma (Tamaki *et al.*, 1976); oviduct (Brown and Badman, 1962; Solomon, 1973; Yamada, 1973; Aire and Steinbach, 1976; Darshan and Panda, 1987); ovary (Chapeau *et al.*, 1996); testes (Gunawardana, 1985; Gunawardana and Viranjanie, 1990); chondrocytes (Wuthier *et al.*, 1985; Reilly *et al.*, 2005); and embryonic stem cells (Yang and Petitte, 1994; Pain *et al.*, 1996).

Previous descriptions of the presence or absence of AP activity in the epithelia of the mature chicken uterovaginal junction (UVJ), sperm-storage tubules (SST) and vagina were contradictory. For example, Fujii (1963) found no AP activity in the UVJ or SST epithelia. In contrast, Aire and Steinbach (1976) observed AP activity in the SST and vaginal epithelia of mature chickens. Given these discrepancies as well as the general lack of knowledge regarding the nature and impact of epithelial cell secretions on sperm transport and selection in the vagina and storage in the UVJ, we examined the distribution of AP in the vagina and UVJ in turkey hens in egg production.

MATERIALS AND METHODS

All turkey hens (Large White commercial breeders) in our breeder flock were maintained under standard husbandry conditions. Eight breeder hens, 34 to 44 weeks of age, with a hard-shell egg in their uteri were euthanised by cervical dislocation. The uterus and vagina were excised and their enveloping connective tissues removed until the vagina was uncoiled and straight. After cutting longitudinally through the uterus and vagina the surface mucosa was

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flooded with saline, scraped free of the underlying muscularis and transferred to a wax-coated dish, stretched to diminish the height of the folds and pinned (Brillard and Bakst, 1990). The saline was replaced with 10% neutral buffered formalin (NBF) and fixed for 18 to 24 h at 5°C. After rinsing in buffer, whole sheets of distended UVJ and vaginal mucosae were subjected to AP localisation. For examination by light microscopy, pieces of mucosa were excised, placed on a slide and cover-slipped. Images were viewed by bright field or differential interference contrast microscopy using a Zeiss Axioskop Microscope.

Localisation of the alkaline phosphatase

The BCIP/NBT Alkaline Phosphatase Substrate Test (Vector Laboratories, Catalogue No. SK-5400) was used for the histochemical localisation of AP reactivity in the UVJ and vaginal mucosae. The BCIP/NBT (bromochloroindolyl phosphate-nitro blue tetrazolium) substrate working solution and Tris–HCl (pH 9·5) buffer were mixed immediately before use and added to the sections. This solution remained on the sections until a suitable dark blue to violet staining developed, generally around 20 to 30 min at room temperature. Sections were washed in buffer for 5 min then rinsed in tap water. For permanent mounting, the sections were dehydrated, cleared and mounted with VectaMount (Vector Laboratories, Catalogue No. 14-5000).

RESULTS

Squash preparations of fixed whole mounts of the vaginal mucosa revealed strong AP activity in randomly scattered surface epithelial cells (Figures 1 and 2). While the primary and secondary mucosa folds were flattened during the fixation process, numerous parallel surface grooves were apparent throughout the length of the vagina (Figures 1 and 2). These surface grooves, which were shallow but as long as 2 mm, were lined with strongly AP reactive cells further highlighting their presence. Occasionally, between these grooves were single examples or clusters of two to 5 pleomorphic invaginations of the surface epithelium that were also lined by strongly AP reactive cells (Figure 2). These were reminiscent of SST but clearly not within the UVJ. Such surface invaginations were widely scattered and have yet to be seen in paraffin sections of vaginal tissue. Similar AP reactivity of the surface epithelium was observed at the UVJ. The most salient feature of the UVJ with respect to AP activity was the SST reactivity. In whole mounts the luminal surfaces of individual SST

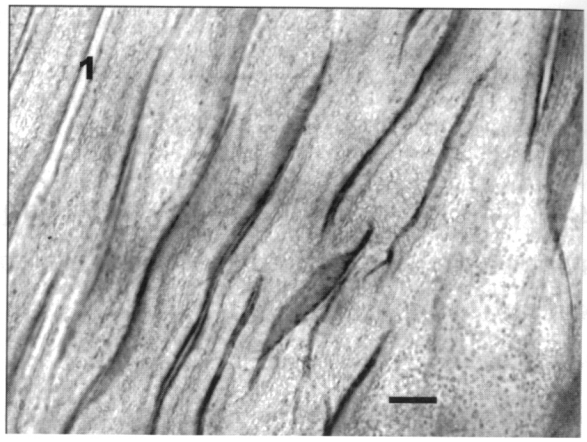

Figure 1. *The mid-portion of the vaginal mucosa revealing AP reactive cells (black) distributed throughout the surface epithelium. The epithelium forming the longitudinal grooves is more densely populated with AP reactive epithelial cells. Bar equals 60 μm.*

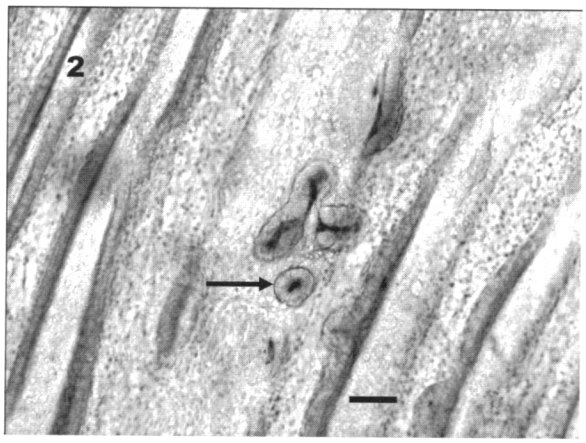

Figure 2. *As in Figure 1, the mid-portion of the vaginal mucosa revealing AP reactive cells (black) distributed throughout the surface epithelium. However, epithelium forming the longitudinal grooves is more densely populated with AP reactive epithelial cells. The arrow indicates AP reactivity associated with the basement membrane of the epithelium forming the surface invaginations. Bar equals 60 μm.*

were strongly AP reactive and obliterated the SST lumen (Figure 3). Paraffin sections confirmed what was suggested in the whole mounts, that is, the apical surface of the SST columnar epithelial cells were strongly AP reactive, while its basement membrane exhibited faint AP reactivity (Figure 4).

DISCUSSION

This is the first report of the presence of AP reactive cells in the oviduct of the mature breeder turkey hen. Previous work has been limited to the chicken and Japanese quail. In these birds, AP activity was present in the regions thought to be involved in cell

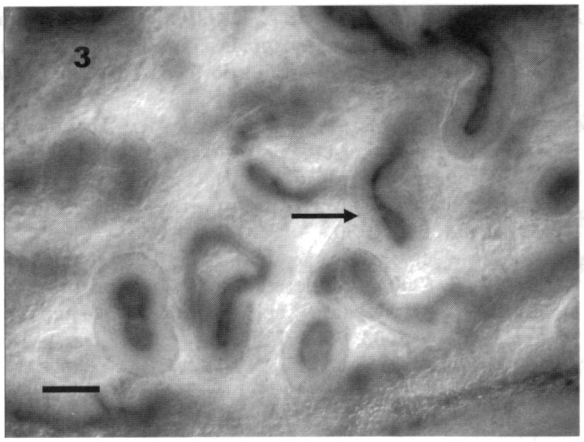

Figure 3. *A whole mount of the UVJ mucosa highlighting several SST possessing a strongly AP reactive epithelium that appears to be occluding their lumina. The arrow indicates less intense AP reactivity associated with the basement membrane of the SST epithelium (arrow). Bar equals 60 μm.*

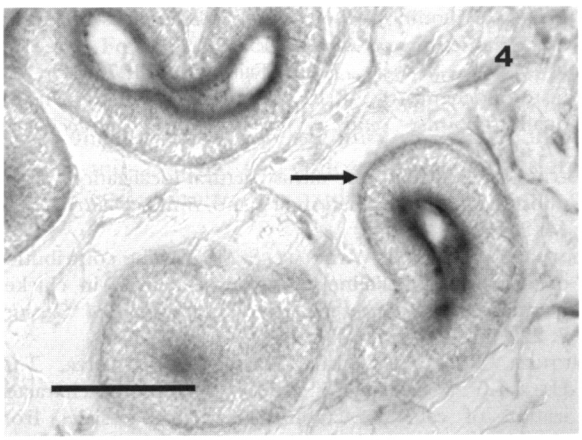

Figure 4. *A histological section of the UVJ revealing SST. In sections, it is clear that the AP reactivity of the SST epithelium is restricted to its apical border and to a lesser extent, its basement membrane (arrow). Bar equals 60 μm.*

differentiation and proliferation during oviduct maturation (Aire and Steinbach, 1976). Interestingly, these are the areas of apposed (closely adjacent) folds and surface invaginations, most likely the initial epithelial budding zones of the tubular glands in segments anterior to the vagina. When the tubular glands were differentiated and surface epithelium consisted of ciliated and secretory cells, AP activity was no longer detectable except in the vagina, UVJ and SST. Consequently, Aire and Steinbach (1976) suggested that AP activity was associated with the cyto-differentiation of the maturing oviduct. Its role in the vagina and SST function was not addressed. Interestingly, the surface grooves observed in the vagina which can be viewed as apposed folds in the mucosal, were AP reactive. Aire and Steinbach (1976) noted that similar AP activity was found in the apposed folds in the immature oviduct anterior to the UVJ.

Sinowatz *et al.* (1976) only observed AP activity in the SST in Japanese quail when an egg was transported through the UVJ. Similarly, we observed intense AP reactivity of the turkey SST epithelium within 3 h of oviposition. However, until more work is completed, one should not associate the observed intense AP activity in the turkey SST with a particular stage of the daily ovulatory cycle.

The role of AP in the SST epithelium may be associated with a transport function or one signalling cell differentiation or proliferation. We know very little about SST formation and proliferation of the SST epithelium. Unlike the tubular glands observed in the distal infundibulum, magnum, isthmus and uterus, SST formation commences prior to the onset of egg production in several avian species and appears not to be oestrogen and/or progesterone dependent (see Bakst, 1988). Histologically, the simple columnar non-secretory epithelium forming individual SST may be viewed as an undifferentiated epithelial cell type, that is, neither a secretory nor a ciliated cell. Its only distinguishable feature is the abundant lipid droplets, possibly having some role associated with resident sperm subsistence (Bakst *et al.*, 1994). The presence of SST in 18-week-old turkey oviduct under non-stimulatory light conditions (Bakst, 1988) and their concurrent differentiation and proliferation with the growth of the oviduct induced by photostimulation suggests that SST epithelial cells may continue to differentiate and proliferate in number while the hen is in egg production. If this hypothesis is correct then the presence of AP in the SST epithelium is consistent with its association with differentiating and proliferating cells. Likewise, the nearly chequered pattern of AP reactive and non-reactive epithelial cells in the vagina may represent sites of AP reactive cell differentiation and proliferation similar to that observed in the immature oviduct.

The strong AP reaction in the SST epithelium may be associated with the transfer of lipid to the SST lumen. Liposome-like vesicles pinching off the apical microvilli of the SST epithelial cells may interact with resident sperm (Bakst, 1993; Bakst *et al.*, 1994). Interestingly, particle bound rat intestinal AP has been implicated in the regulation of lipid transfer across enterocyte cell brush border membranes (Mahmood *et al.*, 1994; Narisawa *et al.*, 2003). We suggest that a similar AP-associated mechanism localised to the apical cytoplasm and microvilli of the SST epithelium may provide lipid for sperm metabolism as well as sperm cell membrane integrity (Bakst, 1993; Bakst *et al.*, 1994).

In conclusion, AP is highly reactive in individual SST and to a lesser extent in the UVJ and vaginal epithelia in turkey hens in egg production. We suggest that AP possibly reflects cell proliferation in the SST and surface epithelia and cell proliferation and lipid transfer activities in the SST. A comparative study examining AP activities and the distribution of cell proliferation markers in the vagina, UVJ and SST epithelia at different stages of the daily ovulatory cycle in hens at the onset of egg production and after 20 weeks of egg production would clarify some of the observations presented in this study.

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