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# Histological Changes in the Epithelium of Thai Swamp Buffalo Oviduct at Follicular and Luteal Phases

Paisan Tienthai<sup>1\*</sup> Kriengyot Sajjarengpong Mongkol Techakumphu

## Abstract

The samples from the infundibulum, ampulla, isthmus and uterotubal junction (UTJ) of the Thai swamp buffalo oviduct were taken immediately after slaughter at the local abattoir. The histological changes of the epithelium including general characteristics, intensity of periodic acid-Schiff (PAS) staining, cell height and immune cell distribution were investigated by light microscopy. The degree of the histological and morphometric changes was shown to be high in the ampulla and infundibulum compared with that of the isthmus and UTJ at both follicular and luteal phases of the estrous cycle. In the infundibulum and ampulla, cytoplasmic protrusions of the epithelial cells with extruded nuclei were prominent at the luteal phase and disappeared during the follicular phase. However, a strong intensity of PAS reaction was obviously shown in the epithelium of isthmus and UTJ during the follicular phase. The epithelial cell height significantly decreased in the infundibulum and ampulla from the follicular to luteal phases, but not in other regions. The number of intraepithelial leukocytes was significantly different between the stages of the estrous cycle and highest in the infundibulum at the luteal phase. In conclusion, histological observations of Thai swamp oviductal epithelium revealed marked cyclic changes in cellular differences associated with functions of segmental variations.

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**Keywords :** buffalo oviduct, epithelium, estrous cycle, light microscopy

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Department of Anatomy, Faculty of Veterinary Science, Chulalongkorn University, Henry-Dunant Rd., Pathumwan, Bangkok, 10330 Thailand

\*Corresponding author

## บทคัดย่อ

# การเปลี่ยนแปลงทางจุลกายวิภาคศาสตร์ของเยื่อบุท่อ นำไข่กระบือปลักไทยในระยะ ฟอลลิคูลาร์และระยะลูทีล

ไพศาล เทียนไทย\* เกรียงยศ สัจจเจริญพงษ์ มงคล เตชะกำพู

เก็บตัวอย่างท่อ นำไข่ส่วนอินฟันดิบูลัม แอมพูลลา อีสรมัส และรอยต่อระหว่างอีสรมัสกับปีกมดลูกของท่อ นำไข่กระบือปลักไทยจากโรงฆ่าสัตว์ท้องถิ่น เพื่อศึกษาการเปลี่ยนแปลงทางจุลกายวิภาคศาสตร์ของเยื่อบุท่อ นำไข่โดยใช้กล้องจุลทรรศน์แสงสว่าง ซึ่งประกอบด้วย ลักษณะโดยทั่วไป ความเข้มในการติดสี periodic acid-Schiff (PAS) ความสูงของเซลล์เยื่อบุ และการกระจายของเซลล์ในระบบภูมิคุ้มกัน ผลการศึกษาพบว่า การเปลี่ยนแปลงทางจุลกายวิภาคโดยทั่วไปของท่อ นำไข่กระบือปลักมีความแตกต่างระหว่างระยะฟอลลิคูลาร์กับระยะลูทีล การเปลี่ยนแปลงนี้เกิดขึ้นในอินฟันดิบูลัมและแอมพูลลาอย่างชัดเจนเมื่อเปรียบเทียบกับอีสรมัสและรอยต่อระหว่างอีสรมัสกับปีกมดลูก โดยพบการยื่นของเซลล์เยื่อบุท่อ นำไข่สูงขึ้นกว่าเซลล์เยื่อบุปกติในระยะลูทีล ซึ่งลักษณะดังกล่าวไม่พบในระยะฟอลลิคูลาร์ อย่างไรก็ตาม ความเข้มของปฏิกิริยาการติดสี PAS ปรากฏให้เห็นชัดเจนในท่อ นำไข่ส่วนอีสรมัสและรอยต่อระหว่างอีสรมัสกับปีกมดลูกในระยะฟอลลิคูลาร์ ขณะที่เซลล์เยื่อบุท่อ นำไข่มีความสูงที่แตกต่างอย่างมีนัยสำคัญในส่วนอินฟันดิบูลัมและอีสรมัส โดยมีเซลล์เยื่อบุสูงขึ้นมากในระยะฟอลลิคูลาร์และลดลงในระยะลูทีล ซึ่งการเปลี่ยนแปลงเกี่ยวกับความสูงของเยื่อบุไม่พบในอีสรมัสและรอยต่อระหว่างอีสรมัสกับปีกมดลูก สำหรับการกระจายของเซลล์ระบบภูมิคุ้มกันซึ่งเคลื่อนที่ขึ้นมาในเยื่อบุท่อ นำไข่กระบือปลัก มีความแตกต่างอย่างมีนัยสำคัญเกิดขึ้นในส่วนอินฟันดิบูลัมเมื่อเปรียบเทียบกับระยะฟอลลิคูลาร์และระยะลูทีล โดยปริมาณของเซลล์ระบบภูมิคุ้มกันพบสูงที่สุดในอินฟันดิบูลัมระยะลูทีล การศึกษาดังกล่าวนี้สรุปได้ว่า การเปลี่ยนแปลงทางจุลกายวิภาคศาสตร์ของเยื่อบุท่อ นำไข่กระบือปลักไทยพบได้อย่างชัดเจนซึ่งเกิดขึ้นตามระยะของวงรอบการเป็นสัด โดยการเปลี่ยนแปลงนี้สัมพันธ์กับหน้าที่หลักของท่อ นำไข่ในแต่ละส่วนที่แตกต่างกัน

คำสำคัญ: ท่อ นำไข่กระบือปลัก เยื่อบุ วงรอบการเป็นสัด จุลทรรศน์แสงสว่าง

ภาควิชากายวิภาคศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ถนนอังรีดูนังต์ ปทุมวัน กรุงเทพฯ 10330  
\*ผู้รับผิดชอบบทความ

## Introduction

It is clear that buffaloes play an essential role in rural livestock production, particularly in Asia, and factors affecting productivity are of paramount important to agricultural economics in this region (Singh et al., 2000). We have known that reproductive efficiency is the main factor influencing productivity and is hampered in the female buffalo (Madan and Raina, 1984). To improve the buffalo reproduction, better understanding of the cellular differences in relation to the functions that occur in the female reproductive tract, particularly in the oviduct, throughout the stages of estrous cycle in buffaloes is primarily required.

The oviduct is a considerable organ in mammalian reproduction because the reproductive processes of male and female gametes occurs in the oviduct (Hunter, 1988; Ellington, 1991). The caudal isthmus and uterotubal junction (UTJ) of the bovine oviduct are involved in events of sperm transport, storage and capacitation that need preservation of the motility, viability and fertilizing ability of spermatozoa (Pollard et al., 1991; Lefebvre et al., 1995) whereas the infundibulum and ampulla are involved in the oocyte pick-up, transport, maturation and fertilization (Hunter, 1988). The oviductal epithelium consists of two morphologically distinct cell types, ciliated and secretory or non-ciliated cells (Abe and

Oikawa, 1993). The ciliated cells are implicated in the transportation of oocytes into the oviduct (Odor and Blandau, 1973), while the secretory cells synthesize secretory products composed of nutrients, specific compound proteins and glycoproteins into the lumen to form oviductal fluid for the reproductive process (Suarez et al., 1997; Leese et al., 2001). In ruminants, the pattern of oviductal secretion and ciliary activity also coincides with changes of the estrous cycle (Abe and Oikawa, 1993; Abe et al., 1993, 1999). Furthermore, there are several mechanisms for maintaining an aseptic micro-environment (Ellis et al., 1986) including the mucosal immune systems of intraepithelial lymphocytes (Morris et al., 1986; Jiwakanon et al., 2005) relating to the function of the oviduct.

To better understand the basic structure and physiology of the buffalo oviduct, more information is needed regarding the differences between the segments associated with the stages of estrous cycle. Therefore, the aim of the present study was to investigate the histological changes of the mucosal epithelium (infundibulum, ampulla, isthmus and UTJ) and the immune cell infiltration of Thai swamp buffalo oviduct with reference to the follicular and luteal phases of estrous cycle.

## Materials and Method

### Animals and tissue collection

The sexually mature female buffaloes ( $n = 27$ ) at various ages (2-8 years) were slaughtered at the local abattoir and genital tracts were immediately collected and kept in a cool container ( $\sim 4^{\circ}\text{C}$ ) for 30 min until analysis in a laboratory. The reproductive organs were examined and the ovarian status of the estrous cycle, i.e. follicular and luteal phases, was determined by the morphological appearance of the corpus luteum (Ali et al., 2003). The samples from four different parts including the infundibulum, ampulla, isthmus and UTJ were fixed in 10% buffered formalin. The specimens were stored at  $4^{\circ}\text{C}$  until being embedded in paraffin

using standard procedures, cut in  $5\ \mu\text{m}$  thick sections and mounted on glass slides for hematoxylin and eosin (H&E) and PAS stainings.

### Cytomorphometric and immune cell evaluations

Samples of all collected buffalo oviducts were stained with H&E and PAS to evaluate the general morphological features, the epithelial cell height and intraepithelial immune cell distribution. A light microscope was used with a x40 objective and x10 eyepieces. The height of epithelium was determined by using Motic Image 2.0 Software and a Moticam digital microscopic camera (Motic Incorporation Ltd., Hong Kong). For this measurement, 150 cells in different locations from each region were selected only if the plane of section clearly passed through the cell nucleus, and the section was parallel to the longitudinal axis of the cell and the apex and base of the cell could be easily distinguished (Verhage et al., 1979). Cell counts were performed using an ocular reticule with 25 small squares placed in one eyepiece of the light microscope. At 400x magnification, each length of 25 small squares of the reticule corresponded to  $125\ \mu\text{m}$  of real tissue length; therefore, the area of 25 small squares of the reticule was equal to  $15,625\ \mu\text{m}^2$  of the real tissue area. The cells were counted on the non-overlapping epithelia of at least 50 small squares of all segments in 4 different sections.

### Statistical analyses

Data were statistically analyzed using the SAS statistical package (version 8.0, SAS Institute, Inc., 1998, Cary, NC, USA). The normal distribution of residuals from the statistical models was tested using the UNIVARIATE procedure option NORMAL. Mean differences in numbers of immune cells and cell heights were tested using analysis of variance (Proc MIXED). The statistical model included the fixed effects of the stage (follicular and luteal) and segment (infundibulum, ampulla, isthmus and UTJ), the interaction between stage and segment, and the random effect of buffaloes nested

within stage. The Bonferroni *t*-test was used to compare the least-square means between groups when overall significance for that was found. *p* value  $\leq 0.05$  was considered statistically significant.

## Results

The morphology of the epithelial mucosa in four different segments of the buffalo oviduct regarding the follicular and luteal phases of the ovarian cycle is shown in Figure 1. Two distinct cell types, ciliated and secretory cells were clearly distinguished by light microscopy in the sections stained with PAS. In the follicular phase, ciliated cells were predominating in the infundibulum (Fig. 1a) and ampulla (Fig. 1c). Intense staining at the apical cytoplasm of the secretory cells in the isthmus (Fig. 1e) and UTJ epithelia (Fig. 1g) were displayed very intensely at the follicular phase, whereas the PAS positive cells in the epithelium of other oviductal regions in the follicular (Figs. 1a, c) and luteal phases (Figs. 1b,d,f,h) were not clear and showed individual variations (Table 1). In addition, the characteristics of secretory cells found in the infundibulum (Fig. 1b) and ampulla (Fig. 1d) were slender-shaped and various degrees of protrusion during luteal phase were found. The cytoplasmic protrusions of secretory cells with/without nuclei of these segments were usually extended beyond the tips of the cilia. Except for the PAS positive cells in the follicular phase, no difference in the morphology of the isthmus and UTJ epithelial cells was observed between the follicular and luteal phases.

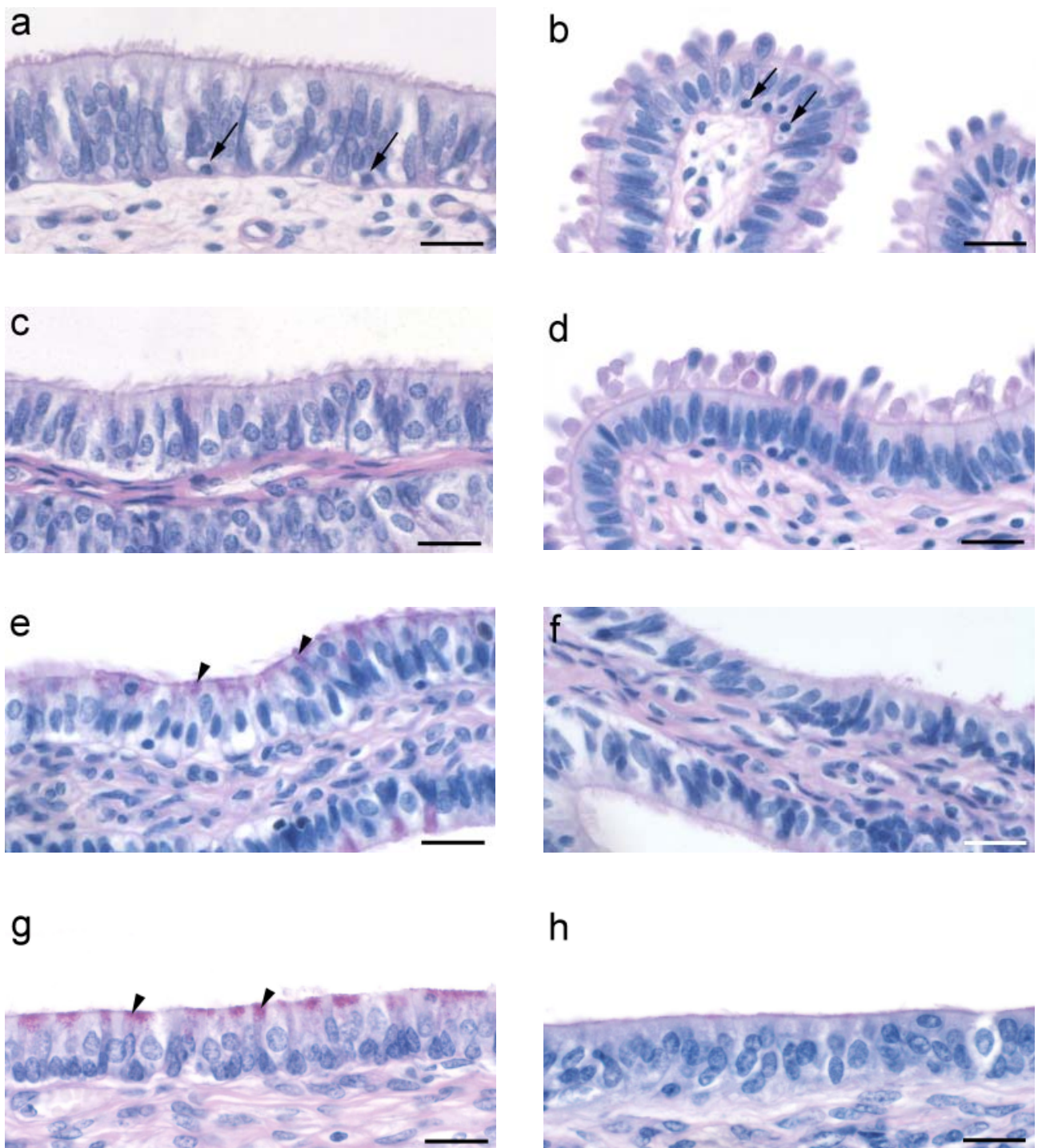
The epithelial cell height and intraepithelial leukocyte distribution of the infundibulum, ampulla, isthmus and UTJ during the follicular and luteal phase are shown in Figs. 2 and 3. The height of epithelium in all segments significantly decreased from the follicular phase to luteal phase and was most dramatic in the infundibulum and ampulla ( $p < 0.05$ ), while this reduction was not significant in the epithelium of the isthmus and UTJ ( $p > 0.05$ ). The characteristics of intraepithelial leukocytes, usually close to basement membrane of the oviductal epithelium, found in the buffalo oviduct were presented as thin, light cytoplasm with large round-shaped nucleus (Figs. 1a,b,d). The numbers of intraepithelial immune cells significantly differed ( $p < 0.05$ ) among segments (Fig. 3) and differed between stages only in the infundibulum (Fig. 4). However, a high variation in the numbers of intraepithelial leukocytes was found among individual buffaloes.

## Discussion

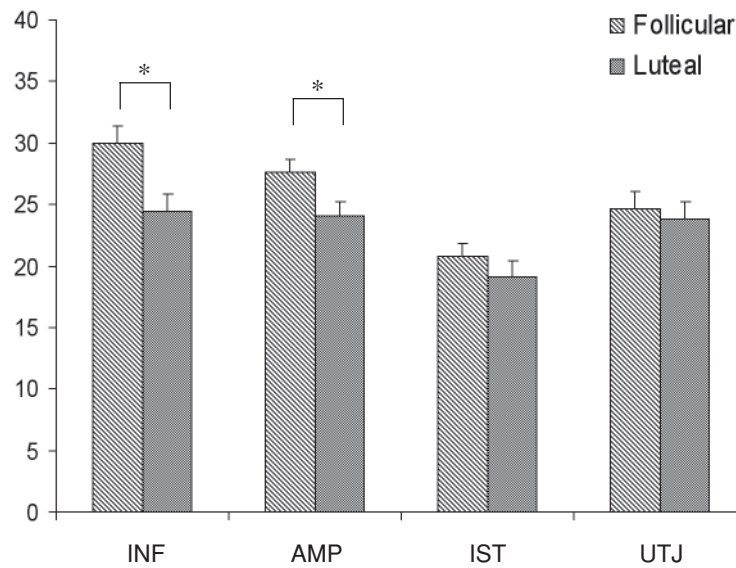
The general histological changes in four different segments of buffalo oviducts during the follicular and luteal phases in this study were relatively similar to the previous observations in the bovine oviduct (Suarez et al., 1997; Bergqvist et al., 2005<sup>a,b</sup>). The secretory cells of the infundibulum and ampulla in the present study dramatically extended beyond the ciliated cells as the bulbous protrusions in the luteal phase, while this phenomenon was not found in the UTJ and isthmus. Murray (1995) and Hollis et al. (1984) who investigated

**Table 1** PAS staining in the epithelial cells of the infundibulum (INF), ampulla (AMP), isthmus (IST) and uterotubal junction (UTJ) at the follicular and luteal phases (+; weak, ++; moderate, +++; intense)

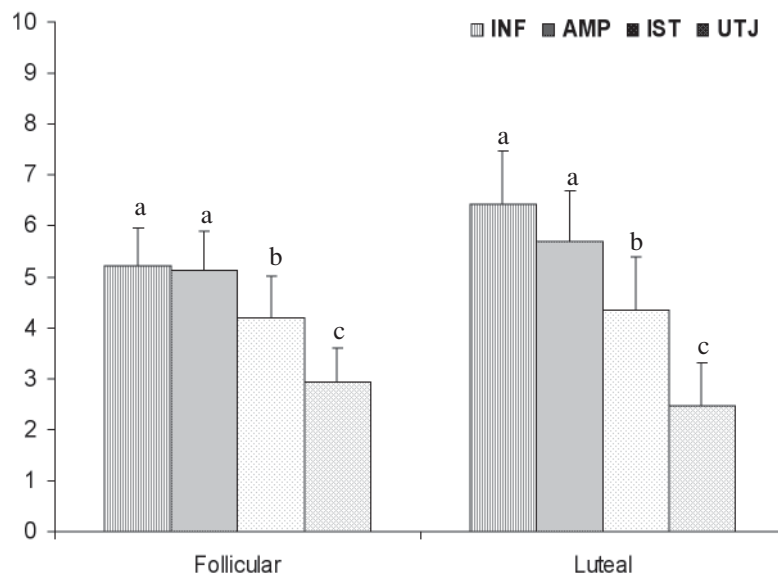
Estrous phase	INF	AMP	IST	UTJ
Follicular	+/++	+/++	+++	+++
Luteal	+	+	+/++	+/++



**Fig. 1** Light microscopic photographs of the buffalo oviduct in cross-section stained by PAS showing the infundibulum (a, b), ampulla (c, d), isthmus (e, f), and uterotubal junction (UTJ) (g, h) at the follicular phase (a, c, e, g) and the luteal phase (b, d, f, h). The apical compartment and surface of non-ciliated secretory cells in the isthmus (e) and UTJ (g) at the follicular phase react with the PAS staining (arrowheads). Notice the lymphocyte-like cells (arrows) are located at the basal compartment of the epithelium. Bars = 20  $\mu$ m.



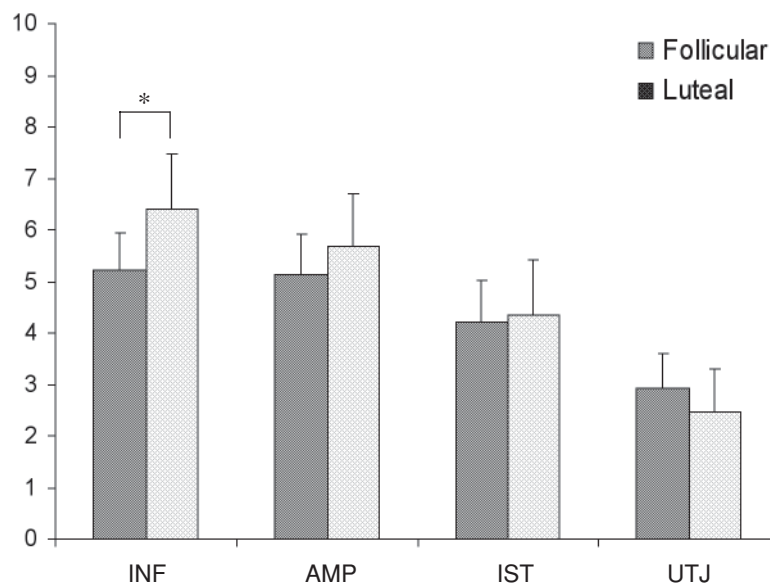
**Fig. 2** The mean percentages of epithelial cell height ( $\mu\text{m}$ ) in the infundibulum (INF), ampulla (AMP), isthmus (IST) and uterotubal junction (UTJ) of the buffalo oviduct at the follicular and luteal phases. Values are presented as means  $\pm$  SEM. “\*” means significant different between follicular phase and luteal phase with  $p < 0.05$ .



**Fig. 3** Distribution of intraepithelial immune cells (number of cells/25 small squares) in different segments of the buffalo oviduct. Values are presented as means  $\pm$  SEM. Bars within the same stage of estrous cycle marked by different letters are significantly different ( $p < 0.05$ ). (INF, infundibulum; AMP, ampulla; IST, isthmus; UTJ, uterotubal junction)

the ewe oviduct suggested that shedding of extruded secretory cells into the oviductal lumen during the luteal phase was a process of cell death. Similar patterns have been seen in sows including the presence of macrophages which normally take care of dead cells in the oviductal epithelium (Jiwakanon et al., 2005). Moreover, the secretory cell in caprine ampullar epithelium showed

entire atrophy, and apoptosis-like fragments were observed in the late luteal phase (Morita et al., 1995). Apoptosis in the oviductal epithelium of cats has been exhibited to be hormonally regulated, induced by a rise of progesterone coupled with a decline in serum estradiol (Bareither and Verhage, 1981). These findings indicate that the cytoplasmic protrusions might be a part of the



**Fig. 4** Distribution of intraepithelial immune cells (number of cells/25 small squares) in the buffalo oviduct at the follicular and luteal phases. Values are presented as means  $\pm$  SEM. “\*” means significant difference between follicular and luteal phases with  $p < 0.05$ . (INF, infundibulum; AMP, ampulla; IST, isthmus; UTJ, uterotubal junction)

process in which dead epithelial cells are eliminated. However, it is very difficult to observe all details in the changes of secretory cells and the macrophage is not recognized in buffalo oviduct by routine histological techniques. Therefore, more special techniques, for example, the immunohistochemical staining of CD68 (Kar et al., 2004) and TUNEL (Gavrieli et al., 1992) might be used for differentiating macrophages and apoptosis along the oviductal epithelium in the near future.

The cytomorphometric data have revealed the tendency of epithelial cell height to be reduced in all segments during luteal phase but this reduction noticeably occurred in the infundibulum and ampulla. Our findings were not exactly similar to those of the previous study in cow oviducts (Abe and Oikawa, 1993) where both ciliated and secretory cells were measured. They reported that the height of ciliated cells significantly decreased in all regions at the luteal phase and were most dramatic in the fimbriae and ampulla as related to the low percentages of these cells in both segments, while the secretory cells decreased in the UTJ and isthmus at luteal phase. Although the present study did not use semi-thin sections stained with toluidine

blue, the measurement of the entire epithelium in the buffalo oviduct can be representative of both ciliated and secretory cells. Therefore, the changes of epithelial cell height found in the buffalo oviduct could be mainly dependent on the presence (ciliation) of ciliated cells at the follicular phase and the loss of ciliated cells (deciliation) at the luteal phase, especially in the infundibulum and ampulla, as it occurs in the rat oviduct (Reeder and Shirley, 1999). Considering the UTJ and isthmus, the results have shown no difference in epithelial cell height between both phases of estrous cycle. As described earlier, the epithelial cell height of the oviduct is involved in the number of ciliated cells. Since the seeming proportion of epithelial cells in UTJ and isthmus showed a little change, the epithelial cell height in these regions did not differ as well. However, the question is why the epithelial cell height in the infundibulum and ampulla was higher than in the UTJ and isthmus. Reeder and Shirley (1999) suggested that epithelial cells in the rat oviduct may transform from one functional cell type to another, which is supported by the fact that ciliated and secretory cells alternately increase and decrease in number without evidence of much mitotic activity in either type. These



mechanisms under the influence of steroid hormones of ciliated and secretory cells in the infundibulum and ampulla could be much more sensitive and functional than these cells in UTJ and isthmus. These findings strongly suggest the regular cycle of ciliogenesis and deciliation by epithelial cells of buffalo oviduct depends on the estrous cycle and reflects the functions in different regions.

The present study showed that the secretory cells in UTJ and isthmus intensely contained the PAS-positive reaction in the apical compartment at the follicular phase as it occurred in porcine (Walter and Bavdek, 1997; Johansson et al., 2000) and bovine (Suarez et al., 1997), while the other regions showed a variation of PAS-staining throughout estrous cycle. Generally, the PAS-positive material detected in the lumen and epithelial cells of the oviduct indicated the presence of mucopolysaccharides and acidic glycoproteins (Oliphant and Ross, 1982) and very high intensity of staining during follicular phase related to the changes in secretory activity under the influence of estrogen (Nayak et al., 1976; Buhi et al., 1996). However, Abe et al. (1993) reported that the intense labeling of oviductal glycoprotein in bovine was observed in the ampullar and fimbriae epithelia at the follicular phase, while the reaction was weaker in the isthmus at both phases. This finding with the variable PAS-positive reactions in infundibulum and ampulla suggested that there would be different in types of glycoprotein or mucopolysaccharide existing in each portion of the oviduct which is important in different functions and segments throughout estrous cycle. For instance, Bergqvist et al. (2005<sup>b</sup>) indicated that hyaluronan (HA) in the secretory fluid was at peak in the isthmus and UTJ at follicular phase and played a role in arresting sperm capacitation and preserving sperm viability. Thus, the appearance of PAS-reaction in these regions might be one of the important factors involved in the formation of functional sperm reservoir in bovine (Lefebvre et al., 1995) and could occur in buffalo.

The intraepithelial leukocyte can be found in all regions of the oviduct in both phases of estrous cycle. The characteristics and position of intraepithelial immune cells in the buffalo oviduct have been related to “type I basal cells” which are identified as the “lymphocyte” and it was the most common immune cell type in the epithelium of the bovine oviduct (Abughrien et al., 2000). It is known that the oviduct of domestic animals has a mucosal immune system which is required for maintaining an aseptic intraluminal fluid (Ellis et al. 1986). Therefore, the presence of intraepithelial lymphocyte (IEL) in the buffalo oviduct might involve in the regulation of local immune response and production of antigen-specific antibodies (Ogra et al., 1994). Quantitative examination by light microscopy revealed that a significant difference in the number of IEL was found among segments of the buffalo oviduct corresponding to the previous study of the bovine oviduct (Abughrien et al., 2000), indicating different regulatory mechanisms of IEL within the oviduct. In the present study, the number of IEL in the UTJ and isthmus was less significantly than that in the infundibulum and ampulla. Although detailed knowledge of the IEL in ruminant oviducts is limited, there are several investigators studying in the sow oviduct. In cyclic sow, the number of IEL did not differ among segments and estrous stages. However, it was interesting to note that the number of lymphocytes in the subepithelial connective tissue of the infundibulum and ampulla was clearly higher than that in the isthmus (Jiwakanon et al., 2005). They indicated that the immune cell reaction had to be low in the isthmus because both spermatozoa, as stored before fertilization, and the semi-allogenic conceptuses could survive. This is exemplified by other immune cells, i.e., neutrophils, which are not present in the isthmus epithelia of cyclic sows (Jiwakanon et al., 2005) or inseminated gilts (Rodriguez-Martinez et al., 1990). The present results also show the tendency of IEL to increase in the luteal phase of all segments, particularly in the infundibulum, in contrast to the numbers of IEL in oviducts of sow

(Jiwakanon et al., 2005) and heifer (Abughrien et al., 2000) that did not significantly differ between estrous stages. It was probably because of differences for technical reasons and individual animals. However, this increase could be explained by the appearance of estrogen and progesterone receptors in the IEL of the human oviduct which fluctuated depending on the menstrual cycle but tended to increase in a progesterone-dependent manner mediating via membrane progesterone receptors (Ulziibat et al., 2006). For better understanding, further studies on the different types of immune cell distribution in different segments of the buffalo oviduct are needed.

In conclusion, the present morphometric studies of the buffalo oviduct revealed a marked change in cellular differences related to the main functions of regional variations throughout the phases of estrous cycle. However, the physiological make-up of buffalo oviductal epithelial cells in the reproductive process needs to be further investigated, especially the localization of female steroid receptors and the presence of glycoproteins and glycosaminoglycans.

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