Original Article

Morphological Studies and Immunolabeling of Female Hormonal Receptors in Thai Swamp Buffalo Oviducts at Prepuberty

Paisan Tienthai* Kriengyot Sajjarengpong

Abstract

The present study aimed to examine the general morphology of Thai swamp buffalo's oviducts by light microscopy (LM) and scanning electron microscopy (SEM) as well as the expression of estrogen receptor alpha (ERα) and progesterone receptor isoform B (PRB) by immunohistochemical technique at the prepubertal stage. The samples composed of the uterotubal junction (UTJ), isthmus, ampulla and infundibulum of prepubertal Thai swamp buffalo oviducts (n=6) were collected from a local slaughterhouse. The results showed that the epithelium of prepubertal Thai swamp buffalo oviducts was composed of ciliated and secretory cells but the feature of nuclear/cytoplasmic protrusions of secretory cells was prominent only in the ampullar and infundibular epithelia. In the UTJ and isthmus, the intensity of PAS staining was reasonably depicted in the secretory cells and the epithelial cell height increased significantly ($p<0.05$) compared to other segments. The distribution of intraepithelial immune cells was demonstrated to be higher ($p<0.05$) in the infundibulum and ampulla than the UTJ and isthmus. The SEM micrographs confirmed the differences between the secretory and ciliated cells as well as the characteristics of the nuclear/cytoplasmic protrusions. The intensity of ER and PR nuclear immunolabeling was stained more significantly ($p<0.05$) in the epithelial cells of the UTJ and isthmus than those of the ampulla and infundibulum. In conclusion, the general histological, SEM and immunolabeling of ER and PR results indicated that there are regional differences to be found in prepubertal Thai swamp buffalo oviducts, i.e. the UTJ and isthmus differing from the ampulla and infundibulum. Indeed, the structures and functional physiology of oviductal epithelial cells might rapidly change for reproduction processes when the ovaries reach to estrous cycle.

Keywords : buffalo, estrogen receptor, morphology, oviduct, prepubertal, progesterone receptor

Department of Anatomy, Faculty of Veterinary Science, Chulalongkorn University, Henry Dunant Rd., Pathumwan, Bangkok, Thailand
***Corresponding Author: Email: Paisan.T@chula.ac.th**

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บทคัดย่อ

การศึกษาสัณฐานวิทยาและการปรากฏของตัวรับฮอร์โมนเพศเมียในท่อนำไข่ของกระบือปลักไทยระยะก่อนวัยเจริญพันธุ์

ไพศาล เทียนไทย* เกรียงยศ สัจจเจริญพงษ์

ศึกษาลักษณะทั่วไปทางสัณฐานวิทยาในท่อนำไข่กระบือปลักไทยโดยใช้กล้องจุลทรรศน์แสงสว่าง และจุลทรรศน์อิเล็กตรอนแบบส่องกราด และการแสดงออกของตัวรับฮอร์โมนเอสโตรเจนอัลฟาและโปรเจสเตอโรนบีโดยวิธีอิมมูนโนฮีสโตเคมีระยะก่อนวัยเจริญพันธุ์ เก็บตัวอย่างท่อนำไข่ซึ่งประกอบด้วยส่วนรอยต่อของปีกมดลูกกับท่อนำไข่ อิสธ์มัส แอมพูลลา และอินฟันดิบูลัมจากกระบือปลักไทยระยะก่อนวัยเจริญพันธุ์จำนวน 6 ตัว จากโรงฆ่าสัตว์ท้องถิ่น ผลการศึกษาพบว่า เยื่อบุของท่อนำไข่กระบือปลักไทยประกอบด้วยเซลล์ชนิดที่มีซีเลียและเซลล์คัดหลั่ง โดยมีลักษณะการยื่นขึ้นมาของเซลล์คัดหลั่งปรากฏชัดเจนในเยื่อบุส่วนแอมพูลาและอินฟันดิบูลัม การย้อมติดสีของ PAS เกิดขึ้นค่อนข้างชัดภายในเซลล์เยื่อบุในส่วนรอยต่อของปีกมดลูกกับท่อนำไข่และอิสธ์มัส ขณะที่เซลล์เยื่อบุมีความสูงมากขึ้นอย่างมีนัยสำคัญทางสถิติในส่วนรอยต่อของปีกมดลูกและอิสธ์มัสเช่นกันเมื่อเปรียบเทียบกับส่วนอื่นๆ ของท่อนำไข่ การกระจายของเซลล์ระบบภูมิคุ้มกันซึ่งเคลื่อนที่ขึ้นมาในเยื่อบุท่อนำไข่กระบือปลักมีความแตกต่างสูงขึ้นอย่างมีนัยสำคัญในส่วนอินฟันดิบูลัมและแอมพูลลา เมื่อเปรียบเทียบกับส่วนรอยต่อของปีกมดลูกกับท่อนำไข่และอิสธ์มัส การศึกษาด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด เป็นวิธีการยืนยันสัดส่วนการกระจายและลักษณะทั่วไปของเซลล์คัดหลั่งและเซลล์ที่มีซีเลีย โดยเฉพาะลักษณะการยื่นของเซลล์คัดหลั่งในส่วนแอมพูลลาและอินฟันดิบูลัมของท่อนำไข่กระบือปลักระยะก่อนวัยเจริญพันธุ์อย่างชัดเจนในการย้อมทางอิมมูนโนฮีสโตเคมีเพื่อตรวจสอบการติดสีของตัวรับเอสโตรเจนและโปรเจสเตอโรน พบการติดสีที่เข้มอย่างมีนัยสำคัญในนิวเคลียสของเซลล์เยื่อบุของส่วนรอยต่อของปีกมดลูกกับท่อนำไข่และอิสธ์มัส เมื่อเปรียบเทียบกับส่วนแอมพูลลาและอินฟันดิบูลัม การศึกษานี้สรุปได้ว่า ลักษณะทางเนื้อเยื่อวิทยาและทางจุลทรรศน์อิเล็กตรอนรวมทั้งการแสดงออกของตัวรับฮอร์โมนเอสโตรเจนและโปรเจสเตอโรน ระบุได้ว่ามีความแตกต่างเกิดขึ้นในส่วนต่างๆ ของท่อนำไข่กระบือปลักไทยระยะก่อนวัยเจริญพันธุ์อย่างชัดเจน นั่นคือ ส่วนรอยต่อของปีกมดลูกกับท่อนำไข่และอิสธ์มัสเปรียบเทียบกับส่วนอินฟันดิบูลัมและแอมพูลลา เป็นที่แน่นอนว่า โครงสร้างและหน้าที่ทางสรีรวิทยาของเซลล์เยื่อบุท่อนำไข่มีการเปลี่ยนแปลงอย่างรวดเร็วสำหรับการทำหน้าที่ในกระบวนการต่างๆ ของการสืบพันธุ์ เมื่อรังไข่เริ่มเข้าสู่วงรอบการเป็นสัด

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ภาควิชากายวิภาคศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330
***ผู้รับผิดชอบบทความ: Email: Paisan.T@chula.ac.th**

Introduction

The mammalian oviducts are very important in several reproductive processes such as the transport of oocytes from the ovaries through the infundibulum and ampulla for fertilization (Ellington, 1991). The caudal isthmus and uterotubal junction (UTJ) are involved in sperm transport, storage and capacitation which are needed to preserve the motility, viability and fertilizing ability of spermatozoa (Pollard et al., 1991; Lefebvre et al., 1995). The epithelial cells of the oviduct consist of ciliated and secretory or non-ciliated cells (Abe and Oikawa, 1993[a]). Indeed, ciliated cells are implicated in oocyte transport (Odor and Blandau, 1973), whereas the secretory cells secrete essential products composed of nutrients, specific compound proteins, glycosaminoglycans and glycoproteins forming intraluminal fluid for fertilization and other reproductive processes (Suarez et al., 1997; Leese et al., 2001; Bergqvist et al., 2005). It has been known that the aseptic environments need to be maintained in the mammalian oviduct to free from microorganisms (Cardenas et al., 1998). Importantly, spermatozoa are allogeneic and therefore, in the oviduct, successful fertilization depends on mechanisms that regulate potentially hostile maternal immune reactions to spermatozoa without hindering

protective immune responses to infectious agents (Jiwakanon et al., 2006).

In a previous paper, the surface epithelium of cycling Thai swamp buffalo oviducts was studied and it was found to be composed of two types of cells similar to other ruminant species, i.e. secretory and ciliated cells, which showed in each segment of the oviduct, marked changes in general histology, cell proportion, epithelial cell height and number of intraepithelial immune cells from the follicular phase to the luteal phase (Tienthai et al., 2008[a]). Essentially, these changes are under the control of estrogen and progesterone which are related to the ovarian status throughout the estrous cycle (Abe and Oikawa, 1993[b]). The influence of these ovarian steroids is mediated in their actions through specific nuclear receptors (King and Greene, 1984) and both the female hormonal receptors are detected in all the oviductal segments of the mature ruminant (Bage et al., 2002; Ulbrich et al., 2003; Valle et al., 2007) including Thai swamp buffalo (Tienthai et al., 2008[b]). However, basic knowledge in the differentiation and functional physiology of the epithelial cells and other compartments of Thai swamp buffalo oviduct need to be acquired in other criteria, for instance, in the neonatal or prepubertal stages. Recently, the differentiation of oviduct epithelium in the bovine calves has been studied and it has been shown that the epithelium of the oviduct also consists of a single columnar epithelium with ciliated cells and protrusions of secretory cells (Kenngott et al., 2008). In addition, estrogen and progesterone receptors have been investigated only in the neonatal oviducts of mice (Greco et al., 1991) and rats (Okada et al., 2003). In addition, the studies involved in the prepubertal oviduct were the essential basic knowledge for the mechanisms of cytodifferentiation and other functions before the animals reaching to the estrous cycle (Sawyer et al., 1984; Verhage et al., 1973). Of course, very little data is available on the general morphological features, the immune cell examination and the immunolocalization of these female hormonal receptors in the oviduct of other species and also in ruminants at the prepuberty.

Therefore, the aim of the present study was to investigate the general histological, cytomorphometric and ultrastructural changes, intraepithelial immune cell infiltration as well as the expression of both estrogen and progesterone receptors in the UTJ, isthmus, ampulla and infundibulum of the Thai swamp buffalo oviduct in relation to the prepubertal stage classified by the ovarian status.

Materials and Methods

Animals and tissue collection

The female genital tract of the prepubertal Thai swamp buffaloes (aged varied between 6 to 8 months, n = 6) were collected from the local slaughterhouse and kept in the cooled container at 4°C for 30-45 min until examination at the laboratory. The reproductive organs were investigated for general normality and both sides of the ovaries were classified into the prepubertal stage by the appearance of numerous small follicles (diameter < 5 mm) without any corpus hemorrhagicum (CH) or corpus luteum (CL) (Fig. 1). The buffalo oviducts were then cut into four segments (UTJ, isthmus, ampulla and infundibulum) and fixed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS; pH 7.4) at 4°C for 24 h for study by conventional scanning electron microscopy (SEM), whereas the other sets of the oviductal samples were immersed in 10% buffered formalin for examination by histological, histochemical and immunohistochemical techniques.

Histochemistry, cytomorphometry and distribution of intraepithelial immune cells

The paraffin-embedded tissues of all the oviductal segments were sectioned (~4-5 μm) and stained with periodic acid's Schiff (PAS) for investigation of the general histology, measurement of the epithelial cell height and the counting of the numbers of intraepithelial immune cells. The epithelial cell height was determined using a light microscope (BX50, Olympus, Tokyo, Japan)

equipped with a digital camera and software program (ImagePro6, Tokyo, Japan) at a magnification 400x. For this measurement, 100 cells in different locations of each segment were selected only if the plane of the section clearly passing through the nucleus and the section was parallel to the longitudinal axis of the cell including the apex and base of the cell could be easily distinguished (Verhage et al., 1979). Immune cell (lymphocytes) counts were performed using an ocular micrometer (ocular reticule) with 25 small squares (15,625 μm^2) placed on the eyepiece of the light microscope as previously described by Tienthai et al. (2008[a]). Counts were performed by the movement of the epithelium in a non-overlapping manner and at least 50 small squares of each segment along the length of the epithelial lining were investigated.

Tissue preparation for routine SEM

After fixing in 2.5% glutaraldehyde, the samples were rinsed in distilled water, post-fixed for 1 h in 1% osmium tetroxide (Merk, Darmstadt, Germany) in PBS (pH 7.4) and washed again. Fixation and washing were carried out at 4°C and the specimens were dehydrated in graded ethanol (30-100%) and substituted with acetone. The tissues were then subjected to critical point drying using liquid CO_2 substitution. Dehydrated samples were mounted on stubs, coated with gold-palladium in a sputter coater, examined and photographed in a JEOL 5800 LV (JEOL, Tokyo, Japan) SEM at the accelerating voltage of 15 kV.

Immunohistochemistry and classification of female steroid receptors

The immunohistochemical procedure has been described earlier by Bage et al. (2002). Briefly, sections were pretreated in a microwave oven at 700 W, in 0.01 M citrate buffer (pH 6.0) for 10 min for antigen retrieval. A standard immunohistochemical technique (avidin-biotin-peroxidase, Vectastain ABC-Elite; Vector Laboratories, Burlingame, CA, USA) was applied to detect the expression of estrogen and progesterone receptors. The primary antibody used was a monoclonal mouse antibody to the estrogen receptor α (ERα, C-311: sc-787; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) at a dilution of 1:50 and a monoclonal mouse anti-chicken PR isoform B antibody (PRB, MAI-411: Affinity Bioreagents, Inc., Golden, CO, USA, diluted 1:250), respectively. The incubation time for the ERα was 1 h at 25°C, whereas the PRB was incubated at 4°C overnight. Negative control was done by replacing the primary antibody with normal mouse IgG (sc-2025; Santa Cruz Biotechnology Inc.). The uterine horn sections from healthy cows known to express both ERα and PRB served as the positive controls. The site of the bound enzyme was visualized by 3,3'-diaminobenzidine in H_2O_2 (DAB kit; Vector Laboratories), a chromogen that produces a brown, insoluble precipitate when incubated with the enzyme. Sections were counterstained with hematoxylin and mounted in glycerol gelatin.

The stained sections were investigated using a LM (BX50, Olympus) equipped with a digital camera and

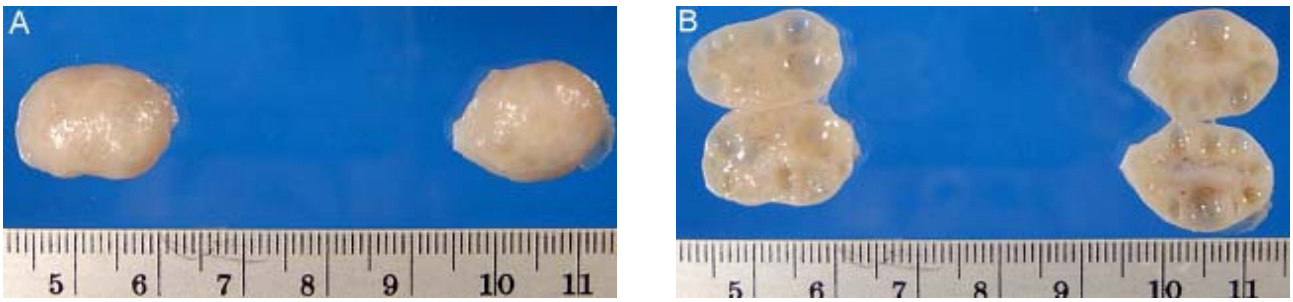

Fig. 1 The appearance of prepubertal Thai swamp buffalo ovaries (A) from the local abattoir showing several small follicles on the surface and the longitudinal sections of both ovaries (B) present the distribution of these follicles in various sizes at the ovarian cortex without any corpus hemorrhagicum and corpus luteum.

software program (ImagePro6). Three different tissue compartments were evaluated separately composed of the layers of epithelium, the subepithelial connective tissue and the smooth muscle. Examination of positive immunolabeling for ERα and PRB was performed by blind preparation. The semiquantitative examination of ERα- and PRB-positive cells was classified at three different levels of intensity in the following staining score criteria: weak, 1; moderate, 2 and strong, 3. Since not all the cells were positive in the three compartments of the oviduct, the proportion of positive to negative cells was estimated for these tissues. The proportions were estimated at four different levels (marked 1-4): low proportion (<30% of positive cells, 1); moderate proportion (30-60% of positive cells, 2); high proportion (>60-90% of positive cells, 3) and almost all cells positive (more than 90%, 4).

Statistical analysis

Data was handled and statistically analyzed using the SAS statistical package (version 8.0, SAS Institute, Inc., 1998, Cary, NC, USA). Differences in the mean numbers of immune cells (lymphocytes) and cell heights were tested using analysis of variance (Proc MIXED). The Kruskal-Wallis test was used to compare the intensity and proportion of ER and PR between segments when the overall significance for that had been found and a p value ≤ 0.05 was considered statistically significant.

Results

Histochemistry, epithelial cell height and immune cell distribution

The general histology of the epithelial layer in all segments of the prepubertal Thai swamp buffalo oviduct is shown in Figure 2. Both ciliated and secretory cells were not clearly distinguished by light microscopy in all sections of Thai swamp buffalo oviducts at prepuberty because there were some of secretory cells that had been detected by PAS staining. However, the secretory cells of the UTJ and isthmus showed more intensity of PAS positive reaction in the apical part than those of the ampulla and infundibulum (Fig. 2 and Table 1). Noticeably, the characteristics of the epithelial cells in the infundibulum (Fig. 2C) and ampulla (Fig. 2D) demonstrated varying degrees of nuclear/cytoplasmic

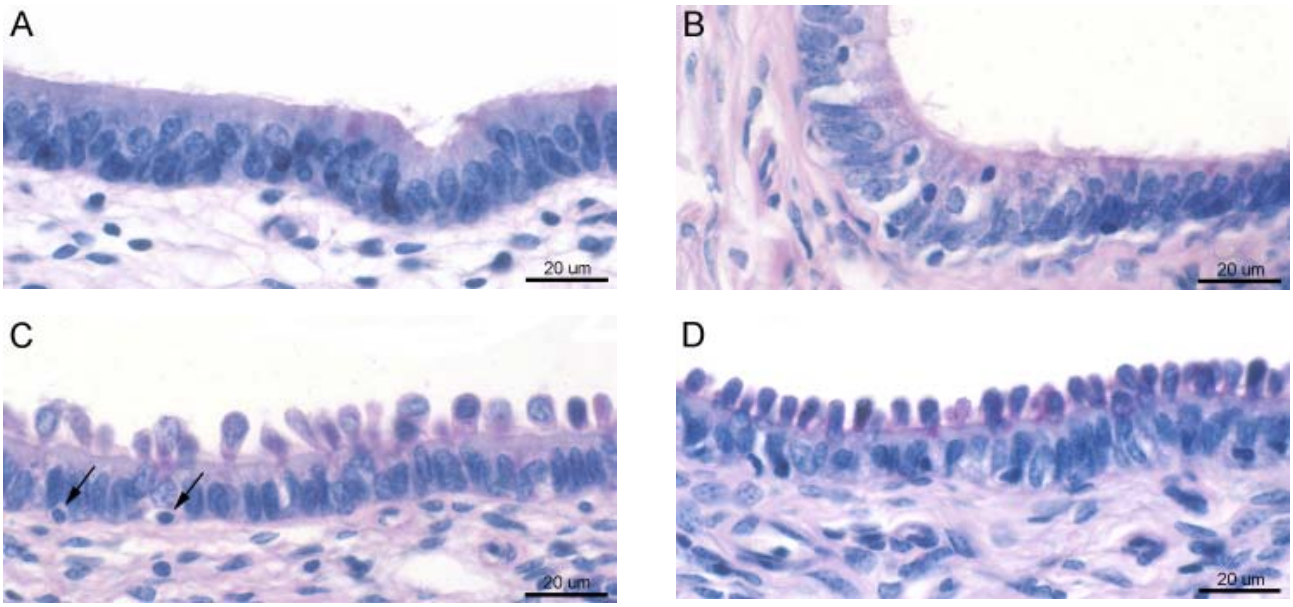

Fig. 2 Surface epithelium of the UTJ (A), isthmus (B), ampulla (C) and infundibulum (D) of prepubertal Thai swamp buffalo stained by PAS. The apical compartment in some of the secretory cells in the UTJ and isthmus fairly reacts with the PAS staining. Notice the nuclear/cytoplasmic protrusions of the epithelial cells in the ampulla and infundibulum and intraepithelial immune cells (arrows) close to the basement membrane of the epithelium. Bars = 20 μm.

Table 1 PAS staining in the epithelial cells of the infundibulum (INF), ampulla (AMP), isthmus (IST) and uterotubal junction (UTJ) of prepubertal Thai swamp buffalo (+; weak, ++; moderate, +++; intense)

Segments/stage	UTJ	IST	AMP	INF
Prepuberty	+/++	+/++	+	+

protrusions which were different from the UTJ (Fig. 2A) and isthmus (Fig. 2B). The nuclear/cytoplasmic protrusions of these segments usually extended beyond the ciliary tips of ciliated cells and some of these protrusions were stained with the PAS staining which was present in the cytoplasm.

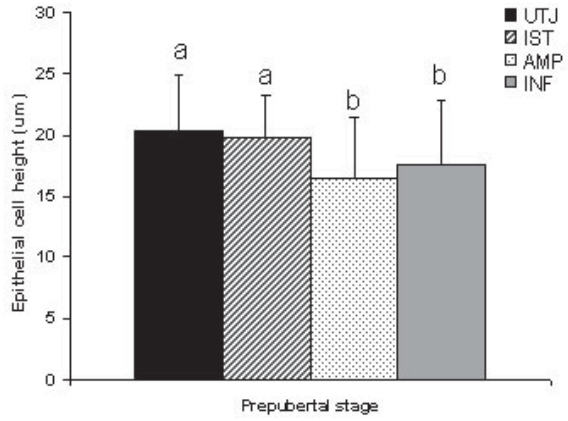

Fig. 3 The mean percentages of epithelial cell height (μm) in the uterotubal junction (UTJ), isthmus (IST), ampulla (AMP) and infundibulum (INF) of the prepubertal Thai swamp buffalo oviduct. Values are presented as mean ± SD with different superscripts being significantly different (p<0.05).

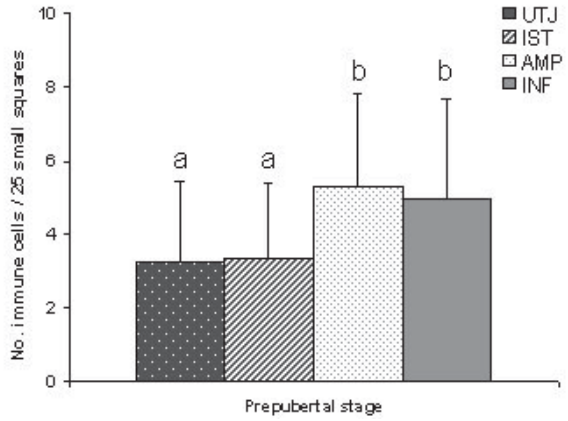

Fig. 4 Distribution of intraepithelial immune cells in the uterotubal junction (UTJ), isthmus (IST), ampulla (AMP) and infundibulum (INF) of the prepubertal Thai swamp buffalo oviduct. Values are presented as mean ± SD with different superscripts being significantly different (p<0.05).

The data on the epithelial cell height and intraepithelial immune cell distribution of the UTJ, isthmus, ampulla and infundibulum in the prepubertal Thai swamp buffalo oviduct is shown in Figs. 3 and 4. The epithelial cells of the UTJ and isthmus were significantly higher (p<0.05) than those of the ampulla and infundibulum. The characteristics of intraepithelial leukocytes found in the prepubertal swamp buffalo oviducts were that they were a thin, light cytoplasm with a large round-shaped nucleus and located at the base of the epithelial layer (Fig. 2C). The numbers of intraepithelial immune cells were significantly lower (p<0.05) in the UTJ and isthmus than the other segments (Fig. 4) of prepubertal swamp buffalo oviduct.

SEM observations

The surface epithelium of the UTJ (Fig. 5A) and isthmus (Fig. 5B) in Thai swamp buffalo at prepuberty examined by SEM clearly showed the differences between ciliated and secretory cells. The ciliated cells of both segments were irregularly distributed on the surface epithelium and their cilia had a variety of lengths, orientations and merged together. However, the apical surfaces of the secretory cells in UTJ were more gently bulging than these cells in isthmus which appeared flat. Importantly, no microvilli or any secretory droplets on the surface of these secretory cells were noticed in either the UTJ or the isthmus as is shown in Fig. 6A. In contrast, the appearance of epithelium in the infundibulum (Fig. 5C) and ampulla (Fig. 5D) by SEM was distinctly different from the UTJ and isthmus because the secretory cells as appearing in the protrusions covered the entire epithelium. The nuclear/cytoplasmic protrusions of the secretory

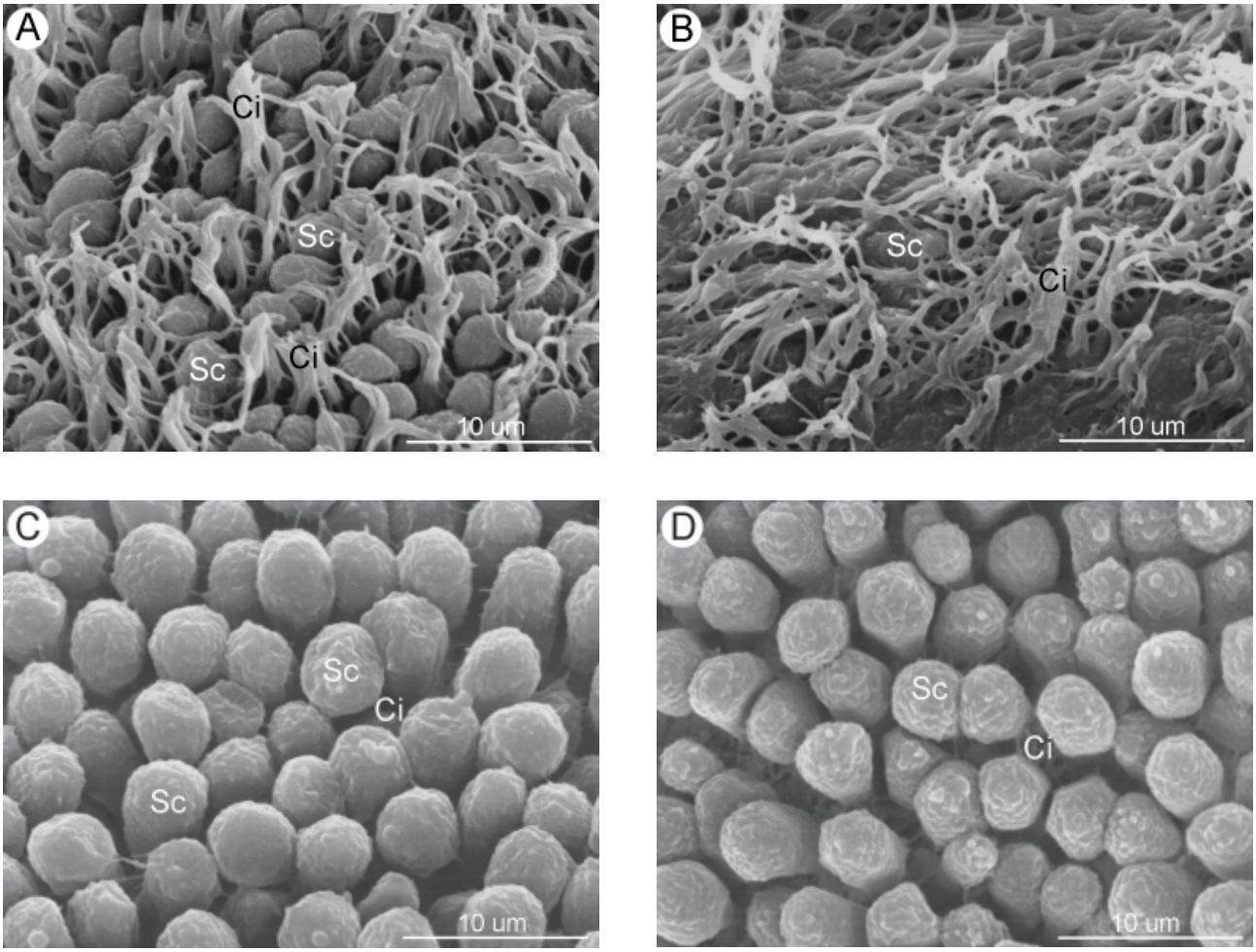

Fig. 5 SEM of the surface epithelium of the UTJ (A), isthmus (B), ampulla (C) and infundibulum (D) in the prepubertal Thai swamp buffalo oviduct. Notice the presence of cytoplasmic protrusions covering the entire epithelium in the ampulla and infundibulum. Ci, ciliated cell; Sc, secretory cell. Bars = 10 μm.

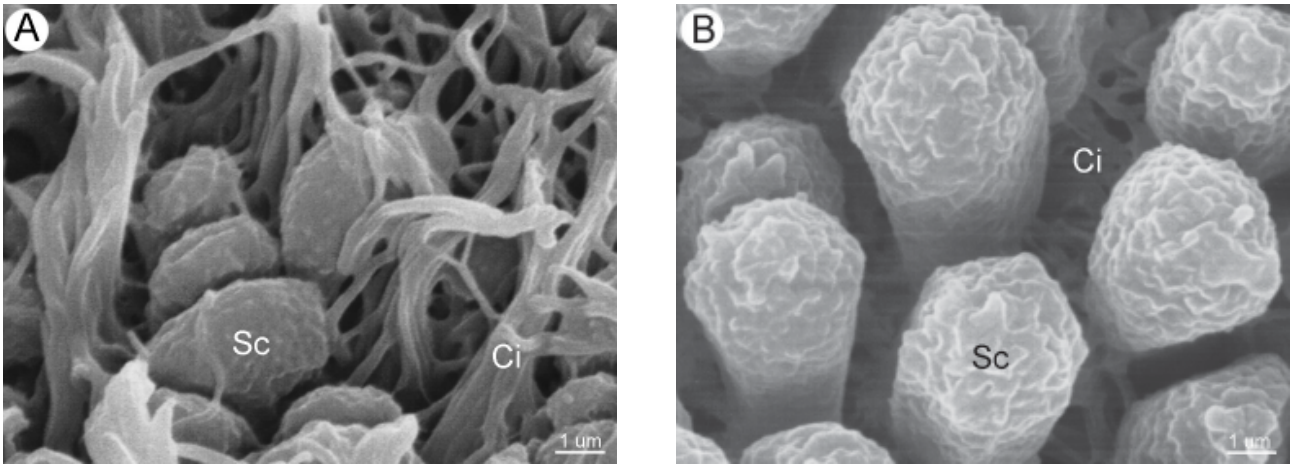

Fig. 6 SEM micrographs of the epithelial cells of the UTJ (A) and infundibulum (B) in the prepubertal Thai swamp buffalo oviduct at higher magnification. Notice the secretory cells without microvilli on the apical surface in the UTJ and infundibulum Ci, ciliated cell; Sc, secretory cell. Bars = 1 μm.

cells were elliptical in shape, uniform in size and showed irregular apical surfaces with no microvilli, whereas the cilia of ciliated cells were hidden by these protrusions (Fig. 6B).

Expression of Female hormonal receptors

ERα and PR isoform B immunolocalization were detected in all compartments of UTJ, isthmus, ampulla and infundibulum of prepubertal Thai swamp buffalo oviducts as depicted in Fig. 7 and 8, respectively. Both ER and PR immunolabeling were stained in the nucleus of epithelial cells, the subepithelial connective tissue cells and the smooth muscle cells but not all the cells in each compartment. Interestingly, ER and PR staining were significantly (p<0.05) shown to be more intense in the epithelium of the UTJ and isthmus than in the ampulla and infundibulum (Figs. 9 and 10). Importantly, the pattern of ER and PR staining was usually demonstrated at the crypts of epithelium in the UTJ and isthmus. In addition, the staining intensity of both ER and PR in the subepithelial and smooth muscular layer was not found to be significantly different (p>0.05) between segments, whereas the immunostaining proportion of both receptors was not shown to be significantly different in any compartment between the segments of the prepubertal Thai swamp buffalo oviduct. However, the tendency for

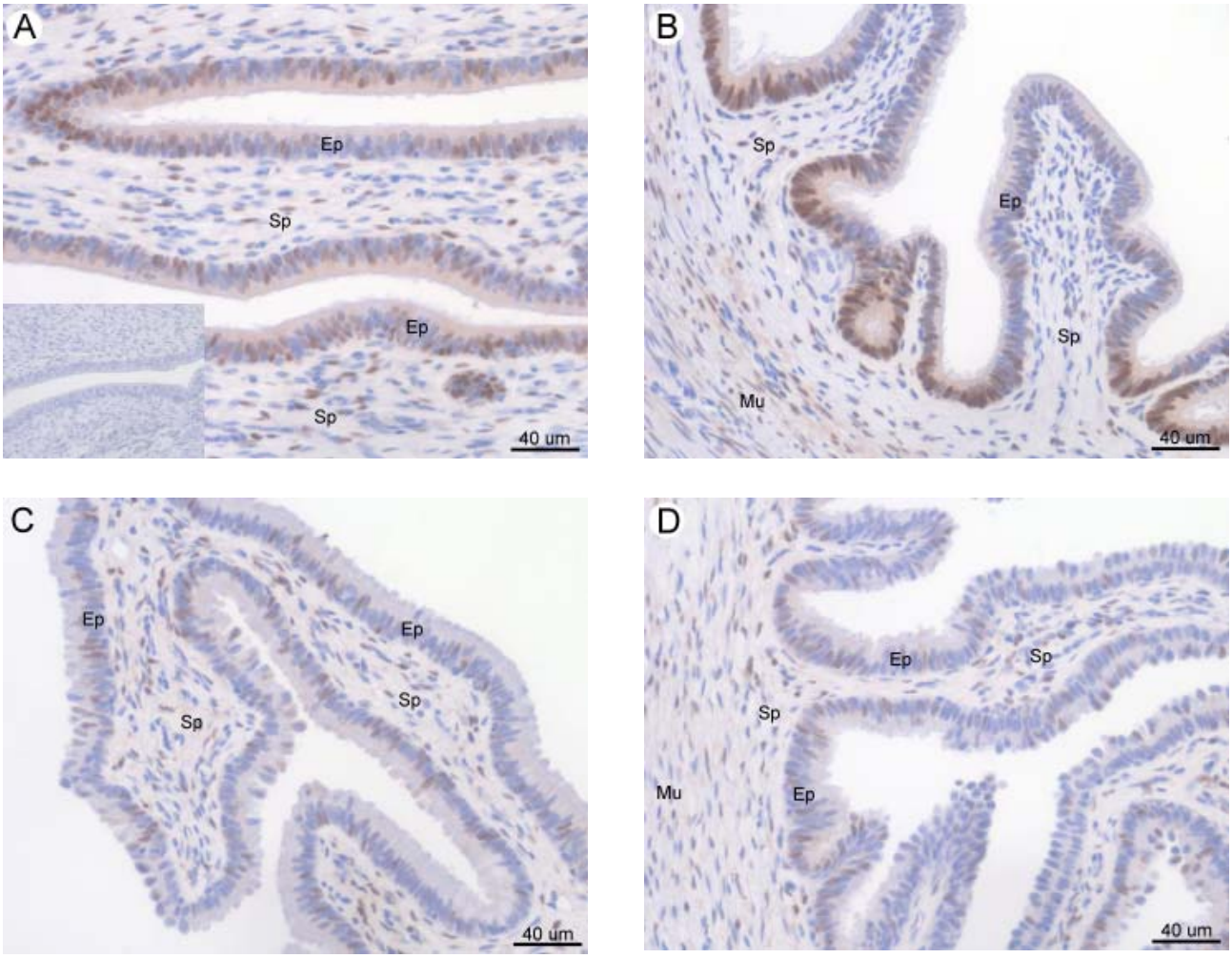

Fig. 7 Immunohistochemical localization of ERα in the epithelium (Ep), subepithelial connective tissue (Sp) and the smooth muscular layer (Mu) of the UTJ (A), isthmus (B), ampulla (C) and the infundibulum (D) of the prepubertal Thai swamp buffalo oviduct. Positive immunolabeling of ERα was stained brown and present in the nuclei. Inset in the UTJ (A) shows the negative control for ERα immunostaining. Bars = 40 μm.

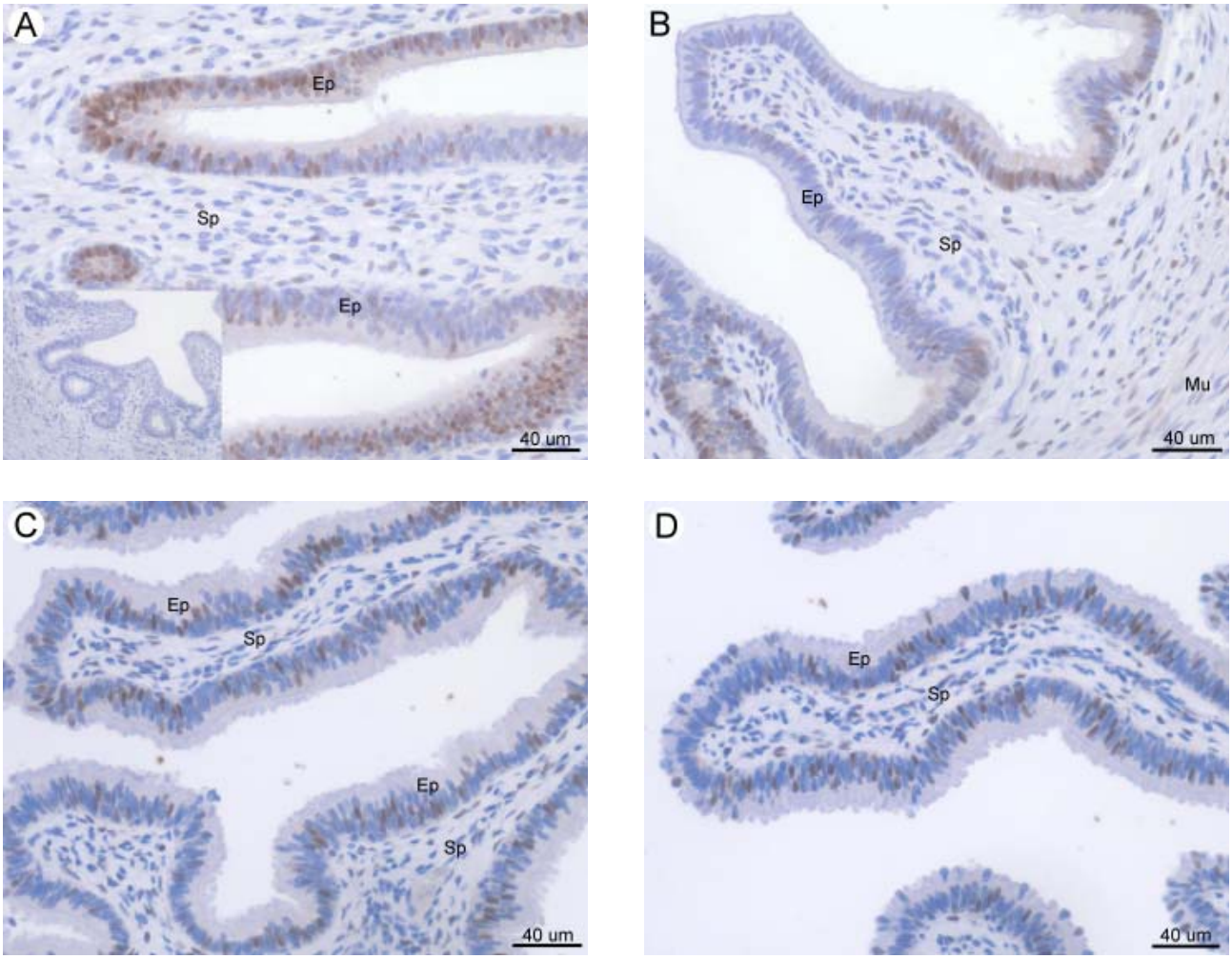

Fig. 8 Immunohistochemical localization of PRB in the epithelium (Ep), subepithelial connective tissue (Sp) and the smooth muscular layer (Mu) of the UTJ (A), isthmus (B), ampulla (C) and the infundibulum (D) of the prepubertal Thai swamp buffalo oviduct. Positive immunolabeling of PRB was stained brown and present in the nuclei. Inset in the UTJ (A) depicts the negative control for PRB immunostaining. Bars = 40 μm.

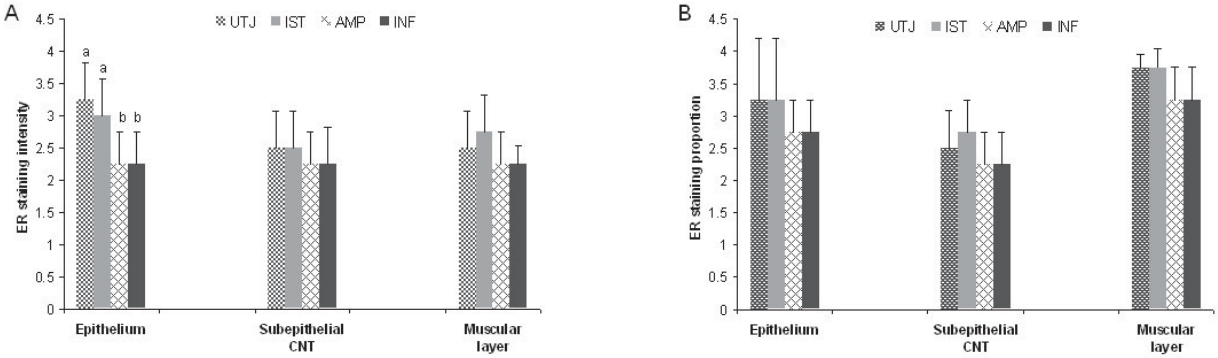

Fig. 9 Intensity (A) and proportion (B) scores of ERα immunolocalization in the epithelium, subepithelial connective tissue and the smooth muscular layer of the uterotubal junction (UTJ), isthmus (IST), ampulla (AMP) and infundibulum (INF) of the prepubertal Thai swamp buffalo oviduct. Values are presented as mean ± SD with different superscripts being significantly different (p<0.05).

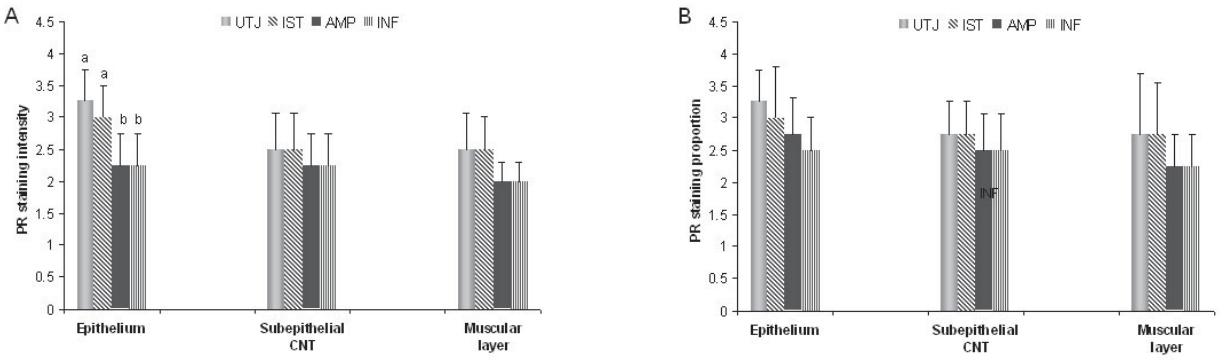

Fig. 10 Intensity (A) and proportion (B) scores of PRB immunostaining in the epithelium, subepithelial connective tissue and the smooth muscular layer of the uterotubal junction (UTJ), isthmus (IST), ampulla (AMP) and infundibulum (INF) of the prepubertal Thai swamp buffalo oviduct. Values are presented as mean ± SD with different superscripts being significantly different ($p<0.05$).

proportion increased in all compartments of the UTJ and isthmus compared with the ampulla and infundibulum (Figs. 9 and 10).

Discussion

General histological changes, including the distribution of intraepithelial immune cells in the oviduct segment of Thai swamp buffalo, have been investigated at the follicular and luteal phases (Tienthai et al., 2008[a]). Previous results demonstrated that the intensity of PAS staining was greater in the UTJ and isthmus corresponding to the results of the present study. The PAS reaction in the apical compartment or apical surface of secretory cells indicated the presence of mucopolysaccharides and acidic glycoproteins (Oliphant and Ross, 1982). Although PAS staining in the UTJ and isthmus in prepubertal Thai swamp buffalo was not shown to be as intense as in mature swamp buffalo (Tienthai et al., 2008[a]), these findings suggest the beginning of secretory activity of the secretory cells in UTJ and isthmus for the further functions such as the forming of a sperm reservoir (Lefebvre et al., 1995). The average of epithelial cell height in UTJ and isthmus found in the cyclic swamp buffalo was 20 to 25 μm (Tienthai et al., 2008[a]) which is a bit higher than these regions of the prepubertal oviduct, whereas the epithelial cell height measured in the ampulla and infundibulum of cyclic swamp buffalo varied from 25 to 30 μm throughout the estrous cycle (Tienthai et al., 2008[a]) and was markedly different from the prepubertal oviduct. It is speculated that epithelial cell changes in height have obviously occurred in the ampulla and infundibulum and these changes suddenly increased when the buffaloes come into the puberty at first estrus under the control of the estrogen level as it occurred in sheep oviducts during the pubertal transition (Lewis and Berardinelli, 2001). When considering the immune cells, the lymphocyte was the most common immune cell type in the cattle (Abughrien et al., 2000; Tienthai et al., 2008[a]) and as we expected the numbers of intraepithelial lymphocyte in the prepubertal oviduct segments were less than the cyclic animals (Tienthai et al., 2008[a]) correlating with the earlier study of Jiwakanon et al. (2005). Additionally, the distribution of these immune cells significantly decreased in the UTJ and isthmus compared with other segments similar to the cyclic buffalo (Tienthai et al., 2008[a]) which supports the regulation of these parts for sperm storage before fertilization as presented in several species (Rodriguez-Martinez et al., 1990; Lefebvre et al., 1995; Suarez et al., 1997).

In the cyclic Thai swamp buffaloes (Tienthai et al., 2008[a]), the oviduct epithelial cells in the UTJ and isthmus studied by LM and SEM showed a few changes between both phases, while the ampulla and infundibulum

appeared to be densely ciliated at the follicular phase. The appearance of cytoplasmic/nuclear protrusions of secretory cells presented at the luteal phase only in the ampulla and infundibulum which is similar to the results in this study which was reported for the first time in prepubertal swamp buffalo oviducts. This phenomenon has been found in different species, particularly in the ampulla and infundibulum while the UTJ and isthmus have not been observed (Walter and Bavdek, 1997; Abe et al., 1999), and have mainly been interpreted in large domestic animals as non-apoptotic signs of cellular degeneration, i.e. cellular turnover (Wrobel et al., 1993), epithelial degeneration (Walter and Bavdek, 1997), or epithelial renewal (Eriksen et al., 1994). In addition, most non-apoptotic degenerative alterations of the secretory cells in cattle have been investigated as progesterone levels physiologically have begun to rise, i.e. the luteal phase, or after progesterone treatment (Nayak and Ellington, 1977; Wrobel et al., 1993; Eriksen et al., 1994). The findings above suggest that the phenomenon of the epithelial cell degeneration can occur in ruminants at all stages, i.e. fetus, prepuberty, luteal phases and pregnancy, which are not only dependent upon the effect of progesterone. Therefore, the cellular physiology of oviduct epithelial cells is now completely unclear and needs further investigation in several respects.

With SEM, Abe and Oikawa (1993[a]) found that the apical surface of the secretory cells in cow oviducts was clearly covered with numerous short microvilli and the cilia of ciliated cells were fairly uniform in length and projected with random orientations into the lumen without merging together corresponding with the secretory and ciliated cells in the Thai swamp buffalo oviduct (P. Tienthai, unpublished observations) and in other species (Abe and Oikawa, 1992; Abe at el., 1993). In contrast to the present data, we found that the apical surface of the secretory cells in all segments of Thai swamp buffalo oviducts demonstrated an irregular surface without microvilli and most of the cilia of ciliated cells were fused together (Figs. 5, 6). The secretory cells of domestic animal oviducts secrete a variety of appropriate substances for the reproductive process occurring in all oviduct segments (Leese et al. 2001). When considering the presence of microvilli, the main function of microvilli is normally absorption, which involves the retention of oviduct fluid throughout the estrous cycle (Hunter, 1988). Therefore, the microvilli in all regions of the oviduct function to regulate fluid in suitable quantities to maintain spermatozoa, oocytes and early embryos in each fertilizing event during transport or contact inside the oviduct. The fewer the numbers and instincts of microvilli on secretory cells in the oviduct will influence the intraluminal environment by the insufficiency of tubal absorption which is found in repeat-breeder heifers (Bage et al. 2002) or in the fetal bovine (Kenngott et al., 2008). In contrast to the secretory cells, the cilia of ciliated cells in the ampulla and infundibulum are considered to be primarily responsible for the pickup and transportation of ovulated oocytes and intraluminal fluid circulation (Hunter, 1988), whereas it is in the cilia in the UTJ and isthmus that spermatozoa are usually believed to bind to maintain their motility, ability and fertilizing capacity (Suarez et al., 1991). Actually, abnormal patterns of cilia have been observed in culling repeat-breeder gilt oviducts (Tienthai et al., 2007). Therefore, the present observations reflect the functional differences in secretory and ciliated cells between the prepubertal and cycling buffalo oviducts indicating the improper function of microvilli and cilia which are not full-grown during prepubertal stage. However, the information involved in the structures of microvilli covering the secretory cells and cilia of ciliated cells in the prepubertal Thai swamp buffalo oviduct requires the examination by TEM in future work.

Up to date, the expression of ER and PR by immunohistochemistry has not been studied in fetal or prepubertal ruminant oviducts, whereas both ER and PR have been detected in the oviducts of mature heifers (Bage et al., 2002; Valle et al., 2007), cows (Ulbrich et al., 2003), sheep (Garcia-Palencia et al., 2007) and

Thai swamp buffalos (Tienthai et al., 2008[b]; P. Tienthai, unpublished observations) during the follicular and luteal phases. As we know the evaluation of the expression and localization of these receptors is a key to clarifying the mechanisms of estrogen and progesterone action on cell proliferation, cytodifferentiation and functional differentiation of the reproductive tissues including the oviduct (Okada et al., 2003). Most authors above have shown that the ER and PR were detected in the nucleus of the cells in all compartments of the oviducts similar to the present work. Furthermore, the intense staining of these receptors varied depending on the segments and phases of estrous cycle in which the ER and PR immunostaining was more intense in the UTJ and isthmus of the Thai swamp buffalo oviduct during the follicular phase (Tienthai et al., 2008[b]; P. Tienthai, unpublished observations). Interestingly, the ER and PR were detected in all segments of neonatal rat oviducts and depicted more intensely in the UTJ and isthmus (Okada et al., 2003). The intensity of both ER and PR found in the present work showed more significantly intensity in the UTJ and isthmus than other segments corresponding to previous researches and this could be explained in that the cellular mechanisms in UTJ and isthmus are more responsive to the stimulation of estrogen and progesterone to function in a different manner than in the ampulla and infundibulum. These findings confirm that the appearance of ER and PR can be detected in the oviduct from the fetal to prepubertal stages in which the ovaries are inactive. In addition, the presence of both receptors may be involved in the cellular turnover, epithelial degeneration or epithelial renewal (Wrobel et al., 1993; Eriksen et al., 1994; Walter and Bavdek, 1997) that occurs as the change in epithelial cell height, secretory activity and cytoplasmic/nuclear protrusions in all oviduct segments of the prepubertal Thai swamp buffalo occur but these mechanisms are still unclear.

In conclusion, the general morphological changes discovered by light- and electron microscope as well as the immunolocalization of ER and PR demonstrated that segmental differences occurred in the prepubertal Thai swamp buffalo oviduct in which all compartments of the oviduct were ready to function under controlling by female hormonal hormones. Additionally, the present study could be helpful for understanding the molecular and cellular mechanisms underlying estrogen and progesterone actions on the Thai swamp buffalo oviduct.

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