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Original Article

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Abstract

Estrogens are known to be an important modulator for regulation of the reproductive function in female mammals. The specific receptor of estrogens in the buffalo reproductive tract, particularly in the oviduct, has not been explored yet in the expression pattern. This study was, therefore, undertaken to investigate the localization of the estrogen receptor alpha (ERα) in the oviduct of Thai swamp buffalo at the follicular and late luteal phases of the estrous cycle. The oviducts were collected from swamp buffalo genital tracts at the local abattoir and separated into the uterotubal junction (UTJ), isthmus, ampulla and infundibulum. Tissue distribution of ERα was examined by immunohistochemical technique and the results showed that ERα was stained in nuclei of cells and can be detected in all compartments along the entire oviduct at both follicular and late luteal phases. The intensity of immunostaining and proportion of ERα positive cells in the epithelium and the subepithelial connective tissue of UJT and the isthmus was significantly higher ($p<0.05$) in the follicular compared to the luteal phase. A higher ERα expression during the follicular phase was also found in the ampulla and infundibulum. However, in these parts of the oviduct the increase in ERα expression was not statistically significant.

Keywords : estrogen receptor, estrous cycle, oviduct, swamp buffalo

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บทคัดย่อ

การปรากฏของตัวรับเอสโตรเจนในท่อนำไข่กระบือปลักไทยระยะฟอลลิคูลาร์และระยะลูเทียล

ไพศาล เทียนไทย[1*] เกรียงยศ สัจจเจริญพงษ์[1] มงคล เตชะกำพุ[2]

ระดับของฮอร์โมนเอสโตรเจน มีบทบาทสำคัญในการควบคุมกลไกและหน้าที่ของระบบสืบพันธุ์เพศเมียในสัตว์เลี้ยงลูกด้วยนม รูปแบบของตัวรับเอสโตรเจนในท่อทางเดินสืบพันธุ์เพศเมียของกระบือโดยเฉพาะในท่อนำไข่ยังไม่เคยมีการค้นคว้าวิจัย ดังนั้น การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาการแสดงออกของตัวรับเอสโตรเจนอัลฟาในท่อนำไข่ส่วนต่างๆ ของกระบือปลักไทยระยะฟอลลิคูลาร์และระยะลูเทียลของวงรอบการเป็นสัด เก็บทางเดินระบบสืบพันธุ์ของกระบือปลักไทยเพศเมียจากโรงฆ่าสัตว์ท้องถิ่น แยกท่อนำไข่ออกจากทางเดินระบบสืบพันธุ์ แล้วตัดแบ่งออกเป็นส่วนรอยต่อระหว่างท่อนำไข่กับปีกมดลูก อิสธ์มัส แอมพูลลา และอินฟันดิบูลัม เพื่อนำไปผ่านกระบวนการทางอิมมูโนฮิสโตเคมีเพื่อตรวจสอบการแสดงออกของตัวรับเอสโตรเจน-อัลฟา ผลการทดลองพบการแสดงออกของตัวรับเอสโตรเจนอัลฟาในนิวเคลียสของเซลล์ และพบได้ในเซลล์ที่ประกอบอยู่ในชั้นต่างๆ ตลอดท่อนำไข่กระบือปลักไทยทั้งระยะฟอลลิคูลาร์และระยะลูเทียล โดยความเข้มและสัดส่วนในการย้อมติดสีบวกของตัวรับเอสโตรเจนอัลฟา ปรากฏชัดเจนอย่างมีนัยสำคัญทางสถิติในชั้นเยื่อบุและชั้นเนื้อเยื่อเกี่ยวพันใต้ชั้นยื่อบุของท่อนำไข่ส่วนรอยต่อระหว่างท่อนำไข่กับปีกมดลูกและอิสธ์มัสในระยะฟอลลิคูลาร์เมื่อเปรียบเทียบกับระยะลูเทียล สำหรับการแสดงออกของตัวรับเอสโตรเจนอัลฟาในท่อนำไข่ส่วนแอมพูลลาและอินฟันดิบูลัม มีการย้อมติดสีบวกที่ชัดเจนมากขึ้นเช่นกันในระยะฟอลลิคูลาร์ อย่างไรก็ตาม ความเข้มและสัดส่วนของการปรากฏในการติสีย้อมดังกล่าวนี้เพิ่มสูงขึ้นอย่างไม่มีนัยสำคัญทางสถิติ

คำสำคัญ : ตัวรับเอสโตรเจน วงรอบการเป็นสัด ท่อนำไข่ กระบือปลัก

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Introduction

The oviduct plays an important role in the critical processes that take place before the establishment of pregnancy in the uterus such as oocyte maturation, sperm reservoir, sperm capacitation, fertilization of gamete and initial embryonic development (Ellington, 1991; Buhi, 2002). Within the oviduct, the gametes and developing embryos contact with the oviductal epithelial cells and their secretions, therefore, intensive research in co-culture experiments has been done in many species including buffalo (Kumaresan et al., 2005; 2006). In ruminants, the oviductal epithelium has been investigated by scanning electron microscopy (Abe and Oikawa, 1993[a]; Abe et al., 1993) and the results demonstrate that there are regional and cyclic changes in the morphological features of the oviductal epithelium. It is well established that these changes are correlated with the varying of estrogen and progesterone blood levels throughout the estrous cycle (Brenner et al., 1974; Abe and Oikawa, 1993[b]). The effects of ovarian steroids are mediated in their actions through specific nuclear receptors (King and Greene, 1984).

Estrogens are considered to be essential for the normal proliferation and differentiation of epithelial cells in the female reproductive tract (Nayak and Ellington, 1977) and appear to regulate the ciliogenesis since ciliated cells are particularly numerous in the bovine oviduct during estrus (Abe and Oikawa, 1993[a]). Maximum secretory cell differentiation is characterized by having the well-developed secretory granules at follicular phase (Nayak and Ellington, 1977). Therefore, the appearance of an estrogen receptor (ER) for this regulation is also required (Greco et al., 1993; Gorski and Hou, 1995).

It is generally accepted that there are different ER subtypes, i.e. ERα and ERβ (Kuiper et al., 1996). It has been found that ERα is predominant in the oviducts of pigs (Nielsen et al., 2001; Steffl et al., 2004), sheep (Garcia-Palencia et al., 2007) heifers (Bage et al., 2002; Valle et al., 2007) and cows (Ulbrich et al., 2003) in which the ERα has been intensely localized in the epithelium during estrus. To our knowledge no comprehensive investigation has been published on the localization of ER in the buffalo oviduct. The aim of the present study was to investigate thelocalization of ERα in different parts of the oviducts collected from Thai swamp buffalo during the follicular and luteal phase of estrous cycle.

Materials and Methods

Animals and collection of tissue samples

The swamp buffalo cows (aged 2-8 years, n=20) were slaughtered at the local abattoir and their genital organs were promptly collected and kept in a cool container at ~4°C for 30 min until being processed in the laboratory. The reproductive organs were examined for normality and the characteristics of the ovarian estrous cycle were classified by changes in the appearance of corpora lutea as described earlier by Ali et al. (2003), i.e. the ovaries at the period of proestrus or estrus were classified as follicular phase (n=8) while the ovaries at late diestrus were classified as luteal phase (n=12). The oviductal samples were cut into four segments, i.e. infundibulum, ampulla, isthmus and uterotubal junction (UTJ), fixed in 10% neutral buffered formalin and embedded in paraffin wax. Thin sections (5μm) were then mounted on Superfrost Plus glass slides (Menzel-Glaser, Freiburg, Germany) and treated according to immunohistochemical procedure.

Immunohistochemical procedure

Before immunohistochemistry, sections were deparaffinized in xylene and rehydrated in graded alcohol. The immunohistochemical protocol has been described previously 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 and allowed to cool for 20 min. A standard immunohistochemical technique (avidin-biotin-peroxidase, Vectastain ABC-Elite; Vector Laboratories, Burlingame, CA, USA) was applied to detect the distribution of the estrogen receptor α (ERα). The primary antibody used was a monoclonal mouse antibody to ERα (C-311: sc-787; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA, dilution of 1:50). The incubation time for the primary antibody was 1 h at 25°C. Negative controls were obtained by replacing the primary antibody with normal mouse IgG (sc-2025; Santa Cruz Biotechnology Inc.) on an equivalent concentration. Normal bovine uterine horn known to express ERα and PRB was served as positive controls. The site of the bound enzyme was visualized by the application of 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.

Classification of ERα-positive cells

Stained sections were evaluated using a light microscope (BX50, Olympus, Tokyo, Japan) equipped with a digital camera (ImagePro6, Tokyo, Japan). Three different tissue compartments were evaluated separately: the surface epithelium, the subepithelial connective tissue and the smooth muscle. Examination of positive immunolabeling for ERα was performed by blinded preparation. The semiquantitative examination of ERα-positive cells was classified to three different levels of intensity in the following staining score criteria: weak, 1; moderate, 2 and strong, 3. Since not all cells were positive in three compartments of uterine tube, the proportion of positive to negative cells was estimated for these tissues. The proportions were estimated into 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 analyses

Data were analyzed using SAS software (Statistics version 9.1 Cary, NC. USA). Descriptive statistics including the mean and standard deviation (SD) of all parameters were calculated. The score of intensities and proportions was compared between segments and phases using NPAR1WAY procedure of SAS (Wilcoxon rank sum test). Differences with $p<0.05$ were regarded as statistically significant.

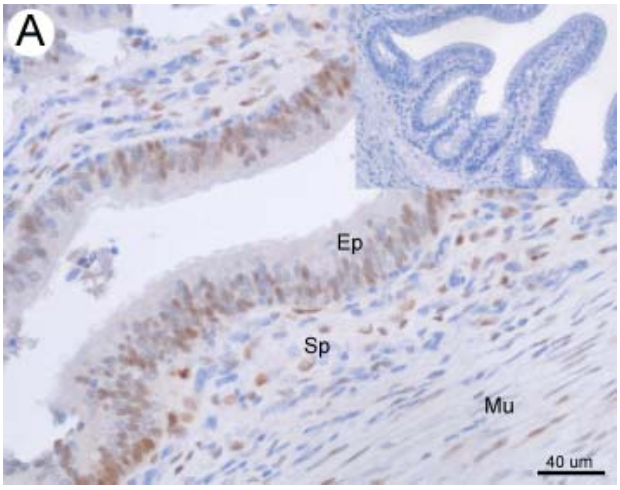
Results

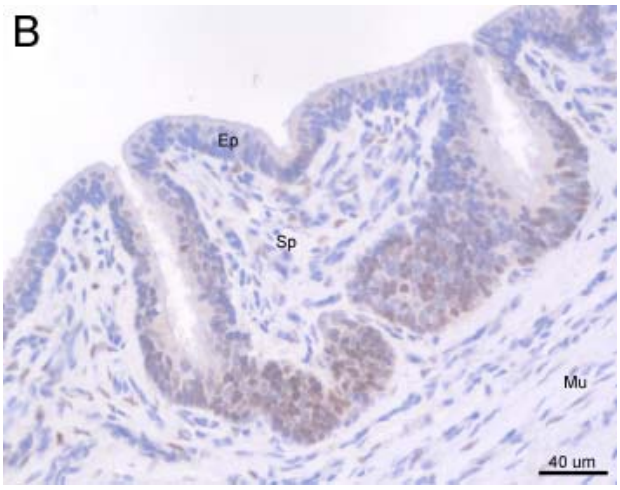
The ERα immunoreactive demonstrated positive nuclear labeling in all tissue compartments and also showed differences between segments and estrous phases as depicted in Fig. 1. Variations were observed with respect to the intensity of the positive staining and regarding proportions of positive/negative nuclei in all oviductal tissue compartments. The semiquantitative results of the positive cells by means of ERα intensity and proportion in the epithelium, subepithelial connective tissue or stroma and smooth muscle are described in Table 1.

In the surface epithelium, the immunohistochemical localization of ERα was obvious in all segments of buffalo oviducts during the follicular phase (Figs. 1A, C, E, G). The inset in Figure 1a demonstrates the negative control for ERα immunostaining. The intensity and proportion of ERα immunoreactivity in the UTJ and isthmus show significantly lower ($p<0.05$) at the luteal phase compared with the follicular phase (Table 1). The distribution of ERα positive labeling in most animals was found to be normally spread out along the surface epithelium of all segments. In the ampulla and infundibulum at the late luteal phase, ERα immunoreaction staining in the bulbous protrusions with nuclei of the epithelium was noticed.

In the subepithelial connective tissue, the ERα localization was stained in the nuclei of undifferentiated connective tissue cells but not all cells (Fig. 1). In addition, the staining intensity and proportion varied depending on the segments, estrous stages and individual animal. However, a stronger intensity and higher proportion were also significantly ($p<0.05$) observed in the UTJ and isthmus at the follicular phase than both parts at the luteal phase.

The smooth muscle nuclei, but not all nuclei, in the muscular layer of swamp buffalo oviducts reacted to ERα immunolocalization as well as the prominent intensity and proportion tended to be greater in the UTJ and isthmus during the follicular phase but the data were not shown any significant differences (Table 1).





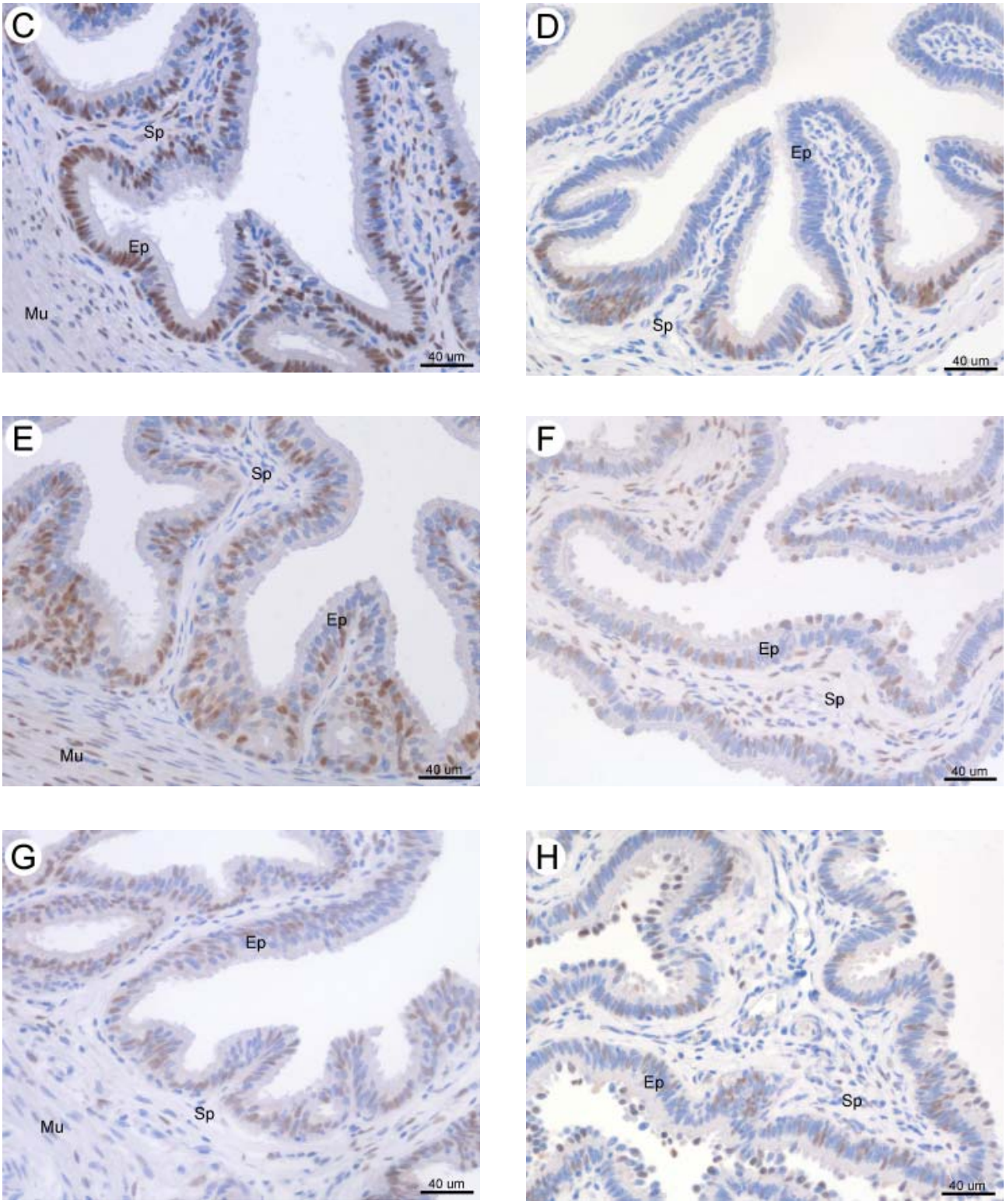


Figure. 1 Immunohistochemical staining of ERα in different compartments of the swamp buffalo oviduct at the follicular phase (A, C, E, G) and luteal phase (B, D, F, H) of the estrous cycle. The ERα nuclear positive cells were stained brown in the epithelium (Ep), subepithelial connective tissue (Sp) and muscular layer (Mu) of UTJ (A, B), isthmus (C, D), ampulla (E, F) and infundibulum (G, H) of buffalo oviduct.

Table 1 Immunohistochemical staining of ERα presented as manual scoring (intensity and proportion as shown by mean ± SD) in different tissue compartments of the Thai swamp buffalo oviduct

Tissue compartments of oviduct/Phases	UTJ	IST	AMP	INF
Surface epithelium				
Follicular	2.6±0.5[a]/2.7±0.9[a]	2.5±0.4[a]/2.7±0.8[a]	2.2±0.6[a]/2.6±1.1[a]	2.2±0.6[a]/2.7±1.2[a]
Luteal	1.5±0.5[b]/1.4±0.6[b]	1.8±0.6[b]/2.0±0.8[b]	1.9±0.6[a]/2.0±0.9[a]	1.9±0.6[a]/2.2±1.1[a]
Subepithelial connective tissue				
Follicular	2.4±0.5[a]/2.7±0.4[a]	2.5±0.5[a]/2.4±0.5[a]	2.3±0.4[a]/2.4±0.7[a]	2.3±0.5[a]/2.6±0.6[a]
Luteal	1.5±0.5[b]/1.7±0.4[b]	1.7±0.7[b]/1.7±0.7[b]	1.7±0.7[a]/1.7±0.6[a]	1.9±0.6[a]/1.9±0.9[a]
Smooth muscle				
Follicular	2.6±0.5[a]/3.6±0.4[a]	2.6±0.5[a]/3.6±0.4[a]	2.4±0.7[a]/3.0±0.5[a]	2.4±0.7[a]/2.8±0.4[a]
Luteal	2.0±0.8[a]/3.0±0.9[b]	2.2±0.9[a]/2.9±0.8[a]	1.8±0.7[a]/2.4±1.1[b]	1.9±0.8[a]/2.2±1.0[a]

The different superscript letters between rows are significantly different ($p<0.05$)
UTJ: uterotubal junction; IST: isthmus; AMP: ampulla; INF: infundibulum

Discussion

The present study is the first to examine the distribution of ERα localization in Thai swamp buffalo oviduct during the follicular and luteal phase of the estrous cycle. The strongest intensity and highest proportion of ERα immunolabeling was particularly detected in the UTJ and isthmus of Thai swamp buffalo oviduct during the follicular phase whereas ERα immunoreaction in the ampulla and infundibulum did not show any differences between phases.

ERα immunoreaction in this study was detected in the nuclei of the luminal epithelial layer as well as nuclear staining of individual stromal and muscle cells which is in agreement with earlier studies of bovine (Ulbrich et al., 2003; Valle et al., 2007), ovine (Garcia-Palencia et al., 2007) and other mammalian species (Press et al., 1986; Amso et al., 1994; Wang et al., 2000; Nielsen et al., 2001). Considering the presence of ERα immunoreaction in the bovine oviductal epithelium, Ulbrich et al. (2003) and Valle et al. (2007) indicated that ERα protein localization in the isthmus and ampulla was detected during all stages of the estrous cycle but was highest in the early luteal phase. In the buffalo, the appearance of ERα in our observations depicted greater nuclear staining in the epithelial cells particularly in the UTJ and isthmus at the follicular phase and weaker at the late luteal phase except for the ampulla and infundibulum. The present data indicate that the UTJ and isthmic epithelium could be the main target of ERα in the swamp buffalo oviduct and its expression may be up-regulated during follicular phase. Furthermore, the ERα localization in the epithelial cells of the UTJ and isthmus could also be correlated with the ERα mRNA transcripts which were elevated during follicular phase as presented in cows (Ulbrich et al., 2003). During the late luteal phase, a weaker staining intensity of ERα expression, investigated in the buffalo oviductal epithelium, was in agreement with previous reports in rats (Wang et al., 2000) and cows (Ulbrich et al., 2003). This may corresponded by several studies suggesting that progesterone down-

regulated ERα expression in target cells in female reproductive organs (Ing et al., 1996; Wu et al., 1996) including in oviductal cell compartments (Garcia-Palencia et al., 2007). To better understanding of the expression of ERα in Thai swamp buffalo oviduct throughout the estrous cycle, the other stages of cycle, especially the early luteal phase, including the plasma levels of estrogens and progesterone need to be done in the future study.

In the subepithelial layer, most of the connective tissue cells were positive at both phases in the present study but the immunostaining tended to be higher during the follicular phase and strongest in the UTJ and isthmus. Kimmins and MacLaren (2001) indicated that estrogen receptors in connective tissue cells enable them to trigger the steroid responsiveness of the epithelium as shown in the study using knock-out mice (Kurita et al. 2000) and different species (Cooke et al., 1997; Robinson et al., 2001). This mechanism could possibly underlie the present results that observed an intense ERα nuclear staining of connective tissue and epithelial cells in the swamp buffalo oviduct. In addition, the up-regulation of ERα during the follicular phase might account for specific compositional changes in buffalo oviductal fluid occurring at this stage as it occurred in the heifer oviducts (Bergqvist et al., 2005). However, there is no report about the trigger mechanism of ERα in connective tissue on the smooth muscle cells therefore this regulation could be investigated.

Regarding the ERα staining intensity and proportion in the muscular layer of the buffalo oviduct, no significant variation was detected corresponding to that reported in heifers (Valle et al., 2007). The ERα immunoreaction in the present study tended to increase during the follicular phase similar to the studies in women's oviducts with time of ovulation (Amso et al., 1994) and sow uteruses at proestrus and estrus (Sukjumlong et al., 2003). To date, there is a little known about the effects of estradiol on the muscular cells in the ruminant oviduct during estrous cycle. The study of sheep oviduct reveals that the characteristics of oviductal motility are consistently increased during estradiol treatment (Ayad et al., 1994) and the motility of porcine oviducts shows high activity at estrous (Rodriguez-Martinez and Einarsson, 1982; Rodriguez-Martinez et al., 1982). Therefore, it is speculated that the contractility of buffalo oviduct at follicular phase may involve in the estrogens which is the one important factor among several factors that function through its receptor in smooth muscular cells.

It is known that a marked change in cellular differences occurs in the ampulla and infundibulum of cow oviducts ascribed by scanning electron microscopy, the epithelium of these regions are densely covered with ciliated cells at the follicular phase while the secretory cells are dominated at the luteal phase (Abe and Oikawa, 1993[a]). In the present study, we have found that the secretory cells of the ampulla and infundibulum extend beyond the ciliated cells as bulbous protrusions during the late luteal phase and this phenomenon was not found in the UTJ and isthmus similar to earlier study (Tienthai et al., 2008). Most authors consider this characteristic in the ampulla and infundibulum as a sign of cellular turnover (Wrobel et al., 1993), epithelial regeneration (Walter and Bavdek, 1997) or epithelial renewal (Eriksen et al., 1994). Previous reports suggest that ERα reactive staining is not well detected in ciliated epithelial cells of the cycling rat oviduct but is expressed in connective tissue stromal, muscle and secretory epithelial cells (Okada et al., 2003). Furthermore, the secretory cells in the UTJ and caudal isthmus of bovine oviduct synthesized hyaluronan and glycoproteins which were essential for the formation of sperm reservoir during estrus phase (Suarez et al., 1997; Bergqvist et al., 2005). This investigation might also support the mechanisms of estrogens and ERα in the specific production of secretory epithelial cells in the UTJ and isthmus of Thai swamp buffalo oviduct. In contrast, the appearance of ERα staining in the secretory epithelial cells in the ampulla and infundibulum may be involved in epithelial cell regeneration for the suitable functions in

these segments via intermediate molecules which are produced by ERα subepithelial connective tissue cells as clearly demonstrated in the mouse uterus (Cooke et al., 1997, 1998).

In conclusion, ERα localization was detected in all compartments of the Thai swamp buffalo oviduct at follicular and late luteal phases in which the ERα positive staining was greater in the UTJ and isthmus during the follicular phase, indicating the different regulations of estrogen mediated its own receptor to fulfill the functions of oviduct associated with the segmental variations throughout the estrous cycle.

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References

Abe, H. and Oikawa, T. 1993[a]. Observations by scanning electron microscopy of oviductal epithelial cells from cows at follicular and luteal phases. Anat. Rec. 235: 399-410.

Abe, H. and Oikawa, T, 1993[b]. Effects on oestradiol and progesterone on the cytodifferentiation of epithelial cells in the oviduct of newborn golden hamster. Anat. Rec. 235: 390-398.

Abe, H., Onodera, M. and Sugawara, S. 1993. Scanning electron microscopy of goat epithelial cells at the follicular and luteal phases of the oestrous cycle. J. Anat. 183: 415-421.

Ali, A., Abdel-Razek, A.K., Abdel-Ghaffar, S. and Glatzel, P.S. 2003. Ovarian follicular dynamics in buffalo cows (Bubalus bubalis). Reprod. Domest. Anim. 38: 214-218.

Amso, N.N., Crow, J. and Shaw, R.W. 1994. Comparative immunohistochemical study of oestrogen and progesterone receptors in the fallopian tube and uterus at different stages of the menstrual cycle and the menopause. Hum. Reprod. 9: 1027-1037.

Ayad, V.J., Gilbert, C.L., McGoff, S.A., Matthews, E.L. and Wathes, D.C. 1994. Actions of oxytocin and vasopressin on oestrogen-induced electromyographic activity recorded from the uterus and oviduct of anoestrous ewes. Reprod. Fertil. Dev. 6: 203-209.

Bage, R., Masironi, B., Sahlin, L. and Rodriguez-Martinez, H. 2002. Deviant peri-oestrual hormone patterns affect the epithelium of the uterine tube in repeat-breeder heifers. Reprod. Fertil. Dev. 14: 461-469.

Bergqvist, A.S., Yokoo, M., Heldin, P., Frendin, J., Sato, E. and Rodriguez-Martinez, H. 2005. Hyaluronan and its binding proteins in the epithelium and intraluminal fluid of the bovine oviduct. Zygote. 13: 207-218.

Brenner, R.M., Resko, J.A. and West, N.B. 1974. Cyclic changes in oviductal morphology and residue cytoplasmic estradiol binding capacity induced by sequential estradiol-progesterone treatment of sprayed rhesus monkeys. Endocrinology. 95: 1094-1104.

Buhi, W.C. 2002. Characterization and biological roles of oviduct specific, oestrogen- dependent glycoprotein. Reproduction. 123: 355-362.

Cooke, P.S., Buchanan, D.L., Young, P., Setiawan, T., Brody, J., Korach, K.S., Taylor, J., Lubahn, D.B. and Cunha, G.R. 1997. Stromal estrogen receptors mediate mitogenic effects of estradiol on uterine epithelium. PNAS. 94: 6535-6540.

Cooke, P.S., Buchanan, D.L., Lubahn, D.B. and Cunha, G.R. 1998. Mechanism of estrogen action: lessons from the estrogen receptor-α knockout mouse. Biol. Reprod. 59: 470-475.

Ellington, J. 1991. The bovine oviduct and its role in reproduction: a review of the literature. Cornell. Vet. 81: 313-328.

Eriksen, T., Terkelsen, O., Hyttel, P. and Greve, T. 1994. Ultrastructural features of secretory cells in the bovine oviduct epithelium. Anat. Embryol. (Berl.) 190: 583-590.

Garcia-Palencia, P., Sanchez, M.A., Nieto, A., Vilar, M.P., Gonzalez, M., Veiga-Lopez, A, Gonzalez-Bulnes, A. and Flores, J.M. 2007. Sex steroid receptor expression in the oviduct and uterus of sheep with estrus synchronized with progestagen or prostaglandin analogues. Anim. Reprod. Sci. 97: 25-35.

Gorski, J. and Hou, Q. 1995. Embryonic estrogen receptors: do they have a physiological function? Environ. Health. Perspect. 103: 69-72.

Greco, T.L., Duello, T.M. and Gorski, J. 1993. Estrogen receptors, estradiol, and diethylstilbestrol in early development: the mouse as a model for the study of estrogen receptors and estrogen sensitivity in embryonic development of male and female reproductive tracts. Endocr. Rev. 14: 59-71.

Ing, N.H., Spencer, T.E. and Bazer, F.W. 1996. Estrogen enhances endometrial estrogen receptor gene expression by a post-transcriptional mechanism in the ovariectomized ewe. Biol. Reprod. 54: 591-599.

Kimmins, S. and MacLaren, L.A. 2001. Oestrous cycle and pregnancy effects on the distribution of oestrogen and progesterone receptors in bovine endometrium. Placenta. 22: 742-748.

King, W.J. and Greene, G.L. 1984. Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. Nature. 307: 745-747.

Kuiper, G.G., Enmark, E., Pelto-Huikko, M., Nilsson, S. and Gustafsson, J.A. 1996. Cloning of a novel receptor expressed in rat prostate and ovary. Proc. Natl. Acad. Sci. 93: 5935-5930.

Kumaresan, A., Ansari, M.R. and Garq, A. 2005. Modulation of post-thaw sperm functions with oviductal proteins in buffaloes. Anim. Reprod. Sci. 90: 73-84.

Kumaresan, A., Ansari, M.R., Garq, A. and Kataria, M. 2006. Effect of oviductal proteins on sperm functions and lipid peroxidation levels during cryopreservation in buffaloes. Anim. Reprod. Sci. 93: 246-257.

Kurita, T., Lee, K., Cooke, P.S., Taylor, J.A., Lubahn, D.B. and Cunha, G.R. 2000. Paracrine regulation of epithelial progesterone receptor by estradiol in the mouse female reproductive tract. Biol. Reprod. 62: 821-830.

Nayak, R.K. and Ellington, E.F. 1977. Ultrastructural and ultracytochemical cyclic changes in the bovine uterine tube (oviduct) epithelium. Am. J. Vet. Res. 38: 157-168.

Nielsen, M., Bogh, I.B., Schmidt, M. and Greve, T. 2001. Immunohistochemical localization of estrogen receptor-α in sex ducts and gonads of newborn piglets. Histochem. Cell. Biol. 115: 521-526.

Okada, A., Ohta, Y., Inoue, S., Hiroi, H., Muramatsu, M. and Iguchi, T. 2003. Expression of estrogen, progesterone and androgen receptors in the oviduct of developing, cycling and pre-implantation rats. J. Mol. Endrocrinol. 30: 301-315.

Press, M.F., Nousek-Goebl, N.A., Bur, M. and Greene, G.L. 1986. Estrogen receptor localization in the female genital tract. Am. J. Pathol. 123: 280-292.

Robinson, R.S., Mann, G.E., Lamming, G.E. and Wathes, D.C. 2001. Expression of oxytocin, oestrogen and progesterone receptors in uterine biopsy samples throughout the oestrous cycle and early pregnancy in cows. Reproduction. 122: 965-979.

Rodriguez-Martinez, H. and Einarsson, S. 1982. Spontaneous contractility of isolated muscle layers of the pig oviductal isthmus. Acta. Physiol. Scand. 115: 325-330.

Rodriguez-Martinez, H., Einarsson, S. and Larsson. B. 1982. Spontaneous motility of the oviduct in the anaesthetized pig. J. Reprod. Fertil. 66: 615-624.

Steffl, M., Schweiger, M. and Amselgruber, W.M. 2004. Immunophenotype of porcine oviduct epithelial cells during the oestrous cycle: a double-labelling

immunohistochemical study. Histochem. Cell. Biol. 121: 239-244.

Suarez, S.S., Blockman, K. and Lefebvre, R. 1997. Distribution of mucus and sperm in bovine oviducts after artificial insemination: the physical environment of the oviductal sperm reservoir. Biol. Reprod. 56: 447-453.

Sukjumlong, S., Kaeoket, K., Dalin, A.-M., Persson, E. 2003. Immunohistochemical studies on oestrogen receptor alpha (ERα) and the proliferative Ki-67 in the sow uterus at different stages of the oestrous cycle. Reprod. Domest. Anim. 38: 5-12.

Tienthai, P., Sajjarengpong, K. and Techakumphu, M. 2008. Histological changes in the epithelium of Thai swamp buffalo oviduct at follicular and luteal phases. Thai J. Vet. Med. 38: 27-37.

Ulbrich, S.E., Kettler, A. and Einspanier, R. 2003. Expression and localization of estrogen receptor alpha, estrogen receptor beta and progesterone receptor in the bovine oviduct in vivo and *in vitro*. J. Steroid. Biochem. Mol. Biol. 84: 279-289.

Valle, G.R., Cassali, G.D., Nogueira, J.C., Castro, A.C., Reis, A.M., Cardoso, F.M., Figueiredo, C.B. and Nascimento, E.F. 2007. Nuclear estrogen and progesterone receptors in the oviduct of heifers under natural and superovulated estrous cycles. Anim. Reprod. Sci. 101: 28-37.

Walter, I. and Bavdek, S. 1997. Lectin binding patterns of porcine oviduct mucosa and endometrium during the oestrous cycle. J. Anat. 190: 299-307.

Wang, H., Eriksson, H. and Sahlin, L. 2000. Estrogen receptor α and β in the female reproductive tract of the rat during the estrous cycle. Biol. Reprod. 63: 1331-1340.

Wrobel, K.H., Kujat, R. and Fehle, G. 1993. The bovine tubouterine junction: general organization and surface morphology. Cell. Tissue. Res. 271: 227-239.

Wu, W.X., Owiny, J., Zhang, Q., Ma, X.H., Nathanielsz, W. 1996. Regulation of the estrogen receptor and its messenger ribonucleic acid in the ovarioectomized sheep myometrium and endometrium: the role of estradiol and progesterone. Biol. Reprod. 55: 762-768.