*Original Article*

# Hyaluronan and Syndecan-1 Localization in the Swamp Buffalo Oviduct

**Paisan Tienthai**

***Abstract***

The glycosaminoglycans (GAGs) found in the mammalian oviductal tissue and luminal secretory fluid influence the fertilization processes in various patterns such as sperm storage, viability and capacitation. The aim of this study was to confirm the appearance of hyaluronan and syndecan-1 in the swamp buffalo oviduct consisting of uterotubal junction (UTJ), isthmus, ampulla and infundibulum during oestrous cycle using histochemical and immunohistochemical techniques. The non-sulphated GAGs, i.e. hyaluronan (HA), were noticeably localized in the subepithelial connective tissue layer or lamina propria of all segments while the epithelial HA-positive staining was demonstrated only in the UTJ and isthmus (sperm reservoir) at the follicular phase. In contrast, the example of the sulphated GAGs, i.e. syndecan-1, were seen along the epithelial lining of all portions and both oestrous stages, however, the syndecan-1 positive labeled on the epithelial lining surface was intensely observed only in the UTJ and isthmus. Therefore, the present results established for the first time that non-sulphated and sulphated GAGs certainly contained in the swamp buffalo oviductal tissue. The noticeable localization of HA in the UTJ and isthmus might indicate the regulation of HA in the forming of sperm reservoir to maintain viability of spermatozoa before ovulation whereas the expression of syndecan-1 may play a vital role in the regulation of sperm adhesion and the initiation of sperm capacitation.

**Keywords:** capacitation, glycosaminoglycans, oviduct, sperm viability, swamp buffalo

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# บทคัดย่อ

## การปรากฏของไฮยาลูโรแนนและซินดิแคน-1 ในท่อนำไข่กระบือปลัก

*ไพศาล เทียนไทย*

ไกลโคสอะมิโนไกลแคนส์ที่พบในเนื้อเยื่อและของเหลวที่คัดหลั่งภายในท่อนำไข่ของสัตว์เลี้ยงลูกด้วยนม มีอิทธิพลต่อกระบวนการปฏิสนธิในลักษณะที่แตกต่างกัน เช่น กลไกการกักเก็บเซลล์อสุจิ การรักษาสภาพมีชีวิตและการคาปาซิเตชันของเซลล์อสุจิ การศึกษานี้จึงมีวัตถุประสงค์เพื่อตรวจหาและยืนยันการปรากฏของไฮยารูแนนและซินดิแคนส์ในท่อนำไข่กระบือปลัก ซึ่งประกอบด้วยรอยต่อของท่อนำไข่กับปีกมดลูก อิสธ์มัส แอมพูลลา และอินฟันดิบูลัมในวงรอบการเป็นสัดโดยวิธีทางฮิสโตเคมีและอิมมูนโนฮิสโตเคมี การปรากฏของไกลโคสอะมิโนไกลแคนส์ชนิดที่ไม่มีซัลเฟตหรือไฮยาลูโรแนน พบได้ชัดเจนภายในชั้นเนื้อเยื่อเกี่ยวพันใต้เยื่อบุ ขณะที่ผลการติดสีบวกของไฮยาลูโรแนนในเยื่อบุพบได้ที่บริเวณรอยต่อของท่อนำไข่กับปีกมดลูกและอิสธ์มัสระยะฟอลลิคูลาร์เท่านั้น ในทางตรงกันข้าม ตัวอย่างการปรากฏของไกลโคสอะมิโนไกลแคนส์ชนิดที่มีซัลเฟต นั่นคือ ซินดิแคน-1 ปรากฏที่เยื่อบุของท่อนำไข่ทุกส่วนและทุกระยะของการเป็นสัด อย่างไรก็ตาม การติดสีบวกที่บริเวณผิวบนของแนวเยื่อบุปรากฏชัดเจนที่บริเวณรอยต่อของท่อนำไข่กับปีกมดลูกและอิสธ์มัส ดังนั้น การศึกษาในครั้งนี้ยืนยันเป็นครั้งแรกว่า ไกลโคสอะมิโนไกลแคนส์ทั้งชนิดที่ไม่มีซัลเฟตและมีซัลเฟต สามารถพบได้ในเนื้อเยื่อท่อนำไข่ของกระบือปลัก การปรากฏอย่างชัดเจนของไฮยาลูโรแนนที่พบบริเวณรอยต่อของท่อนำไข่กับปีกมดลูกและอิสธ์มัส อาจระบุถึงกลไกการทำงานบางอย่างของไฮยาลูโรแนนเพื่อสร้างที่กักเก็บเซลล์อสุจิและเพื่อคงสภาพการมีชีวิตของเซลล์อสุจิก่อนการตกไข่ ขณะที่ การปรากฏของซินดิแคน-1 อาจมีบทบาทสำคัญในกลไกการยึดติดของเซลล์อสุจิกับเยื่อบุท่อนำไข่และกลไกเริ่มต้นของการคาปาซิเตชัน

**คำสำคัญ:** คาปาซิเตชัน ไกลโคสอะมิโนไกลแคนส์ ท่อนำไข่ การมีชีวิตของอสุจิ กระบือปลัก

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## *Introduction*

For the period of natural mating in the cattle, the semen are deposited in the vagina and the spermatozoa rapidly transport through the uterine body, uterine horns and about thousands of them are stored in the uterotubal junction (UTJ) and caudal isthmus, the site served as a sperm reservoir (Hunter and Wilmut, 1984). At the ovulation time, those spermatozoa are subsequently released from the sperm storage, prepare themselves for capacitation and move forward to the segment of fertilization, i.e. the ampullary-isthmic junction (AIJ) (Lefebvre and Suarez, 1996). It is also recognized that the oviductal sperm reservoir sustains viable spermatozoa and preserves sperm fertilizing capacity before the beginning of ovulation (Suarez, 1998; Tienthai et al., 2000). Moreover, there are verifications indicating that the sperm reservoir is implicated in the beginning of the regulation of sperm capacitation that coordinates the sperm membranous changes and the regulation of sperm release (Fazeli et al., 1999; Suarez, 2002). The important functions in the sperm reservoir as mentioned above are mainly correlated to the mechanisms of the sperm-epithelium interactions and the sperm-intraluminal fluids within this region (Suarez, 1998; Rodriguez-Martinez et al., 2001). Among the mucous-like substances appearing in the oviductal secretions, glycosaminoglycans (GAGs) have been localized in the oviducts of several species (Lee et al., 1986; Tienthai et al., 2000; Bergqvist et al., 2005[a]; Bergqvist and Rodriguez-Martinez, 2006) and different kinds of GAGs are responsible in part for the building up of a sperm reservoir (Suarez et al., 1998; Hunter et al., 1995)

The intraluminal secretion within the UTJ and isthmus at pre-ovulatory stage in livestock animals is mucous-like substances depicted positive reaction by Alcian blue and PAS staining (Johansson et al., 2000) which means it is prosperous in glycoproteins and GAGs (Tienthai et al., 2000; Bergqvist et al., 2005[a], Bergqvist and Rodriguez-Martinez, 2006). In general, GAGs are long and no branching polysaccharide chains which are composed of the repeating disaccharide units and two major groups of GAGs are classified into 1) non-sulphated GAGs, i.e. hyaluronan or hyaluronic acid (HA) and 2) sulphated GAGs consisting of chondroitin sulphate, dermatan sulphate, keratin sulphate and heparan sulphate (Lindahl and Hook, 1978). Normally, most of the sulphated GAGs apart from HA found in the tissues take place in a pattern of proteoglycans which are defined as one or more polysaccharide chains appended to a core protein (Kjellen and Lindahl, 1991). The astonishing property of the sulphated GAGs, especially heparan sulphate, is competent to exclusively interact with a group of essential growth

factors and practical proteins (David, 1993). Absolutely, the heparan sulphate transmembrane proteoglycans which are called syndecans play an important role in an assortment of cellular functions, for example the cell proliferation, cell-matrix or cell-cell adhesions and also modulating the activation of cell surface signaling receptors (Zimmermann and David, 1999; Multhaupt et al., 2009; Xian et al., 2010). Syndecans are composed of 4 members, i.e. syndecan-1/syndecan, syndecan-2/fibroglycan, syndecan-3/N-syndecan and syndecan-4/ryodocan (Elenius and Jalkanen, 1994). It was shown that the syndecans could be found in the mammalian oviductal fluids and tissues, however, the syndecan-1 was considered to be a major syndecan that contains in the porcine (Tienthai et al., 2000) and bovine (Bergqvist and Rodriguez-Martinez, 2006) oviducts. It is demonstrated that syndecan-1 might be involved in the sperm capacitation in the bovine oviduct (Bergqvist et al., 2006). Additionally, HA demonstrated the highly viscous condition interacts with cell surface receptors that transduce intracellular signals and affect cellular form and behavior (Toole, 2002). It is believed that signaling pathway of HA and its main receptor CD44 might play a role in the sperm reservoir, e.g. the preservation of sperm viability, fertilizing ability and also functioning as sperm selector to decrease polyspermy (Tienthai et al., 2000; Rodriguez-Martinez et al., 2001).

However, the localization of the HA and syndecan-1 in the swamp buffalo oviductal tissues for understanding their contribution with the gametes, especially spermatozoa during estrous cycle were not yet explored. The present research, therefore, aims to verify the appearance of both types of GAGs, i.e. HA and syndecan-1, in all portions of the swamp buffalo oviduct, which is composed of the UTJ, isthmus, ampulla and infundibulum, during the follicular and mid-luteal phases using histochemistry and immunohistochemistry.

## *Materials and Methods*

***Animal and tissue collections:*** The oviducts and ovaries from mature swamp buffaloes (n=20, ages 3-8 years) were promptly collected post-mortem from local slaughterhouses. All samples were reserved in a cool chamber (~4$^{o}$C) and directly taken to the laboratory within 3-4 hours. The oestrous cycle periods of these swamp buffaloes were identified by the morphological characteristics of the corpus luteum and dominant follicle on the ovaries (Ali et al., 2003) which can be divided into the follicular (n=10) and mid-luteal (n=10) phases. The buffalo oviducts were separated into the uterotubal junction (UTJ), isthmus, ampulla and infundibulum, immersed in 4% paraformaldehyde and routinely embedded in paraffin. All tissue blocks were sliced at 4 µm thickness and the serial cross-sections were placed on Superfrost plus slides (Menzel-Graser, Frieburg, Germany) for histochemical and immunohistochemical procedures.

***Histochemical detection of HA:*** The protocol for HA histochemical localization was thoroughly described earlier by Tienthai et al. (2000). Briefly, the oviductal sections were deparaffinized in xylene and rehydrated in different graded alcohols. The tissue slides were immersed with 3% hydrogen peroxide ($H_2O_2$) in methanol for 10 min at room temperature to hamper endogenous peroxidase activity. After rinsing twice with phosphate buffer saline (PBS, pH 7.4), the sections were applied with 1% bovine serum albumin (BSA) in PBS for at least 30 min to block the non-specific binding. The slides were rinsed again in PBS and then incubated overnight with the biotinylated hyaluronan binding protein (HABP) probe (Seikagaku corp., Tokyo, Japan) at a dilution of 1:100 (10 µg/ml) in PBS at 4$^{o}$C. Bound antibody on the oviductal tissues was detected by the use of avidin-biotin-complex (ABC) reagents at a dilution of 1:100 (Vectastain ABC-Elite, Vector Laboratories, Burlingame, CA, USA) in PBS. All sample sections were reacted with 3, 3′-diaminobenzidine substrate (DAB kit, Vector laboratories) and $H_2O_2$ in distilled water to visualize bound activity. The slides were finally counterstained with Mayer's hematoxylin and mounted in glycerin-gelatin. Negative control slides were performed by replacing biotinylated HABP with 50 units/ml of Streptomyces hyaluronidase (Sigma, St. Louis, MO, USA) at 37$^{o}$C for 4 hours before HABP incubation. The subepithelial connective tissue layer (lamina propria) in each segment of swamp buffalo oviducts was considered to be the positive control as suggested by Edelstam et al. (1991) because this layer contains extracellular matrix which was always positively stained with HA.

***Immunohistochemical detection of syndecan-1:*** After deparaffinization and rehydration, tissue sections were quenched with 3% $H_2O_2$ in methanol and washed in PBS. For the primary antibody, the mouse anti-human syndecan-1 monoclonal antibody (DL-101, sc-12765, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) was used as suggested by Pierce et al. (1992). The tissue sections were applied with normal horse serum at a dilution of 1:10 in PBS for 30 min, prior to incubation overnight at 4$^{o}$C in a humid chamber with a 1:100 dilution of syndecan-1 antibody. For the control slides, the sections were incubated with PBS instead of primary antibody. After rinsing with PBS, all sample slides were applied with the secondary biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA, USA) at a 1:500 dilution for 30 min at room temperature. After rinsing in PBS, the sections were incubated with avidin-biotin-complex (ABC) reagents at a dilution of 1:100 (Vectastain ABC-Elite, Vector Laboratories) for 30 min at room temperature and bound peroxidase was reacted with 3, 3′-diaminobenzidine substrate (DAB kit, Vector laboratories) in $H_2O_2$ for 1-3 min. Negative control slide was made by replacing primary antibody with normal mouse IgG (sc-2025, Santa Cruz Biotechnology) at a dilution of 1:100 while the uterus which is known to express syndecan-1 was served as positive control (Lai et al., 2007).

All slides were counterstained with Mayer's haematoxylin and mounted in glycerin-gelatin. Both histochemical and immunohistochemical stained

slides were observed under a light microscope (BX50, Olympus, Tokyo, Japan) equipped with a digital camera Micropublisher 5.0 (Qimage, Surrey, BC, Canada) and software program (Image-Pro Plus 6.0 (Media Cybernatics Inc., Bethesda, MD, USA).

## *Results*

***Hyaluronan localization:*** As we expected, the HA positive labeling was intensely appeared in the subepithelial connective tissue (lamina propria) layer in the entire segments of the swamp buffalo oviduct at both phases of estrous cycle (Fig 1a-h). It is noticed that the HA appearance was almost unseen in the oviductal lining epithelium except for the UTJ and isthmus, the sperm reservoir, at the follicular phase (Fig 1a & 1c). The pattern of HA localization in the lining epithelium was usually found at the basolateral domain whereas the HA labeling was presently scattered in the supranuclear domain of the oviductal epithelial cells, especially in the epithelial lining crypts of the sperm reservoir. In the control sides, the oviductal tissue sections of pre-incubated with *Streptomyces hyaluronidase* abolished the HA labeling in all compartments of the buffalo oviduct (inset in Fig 1a).

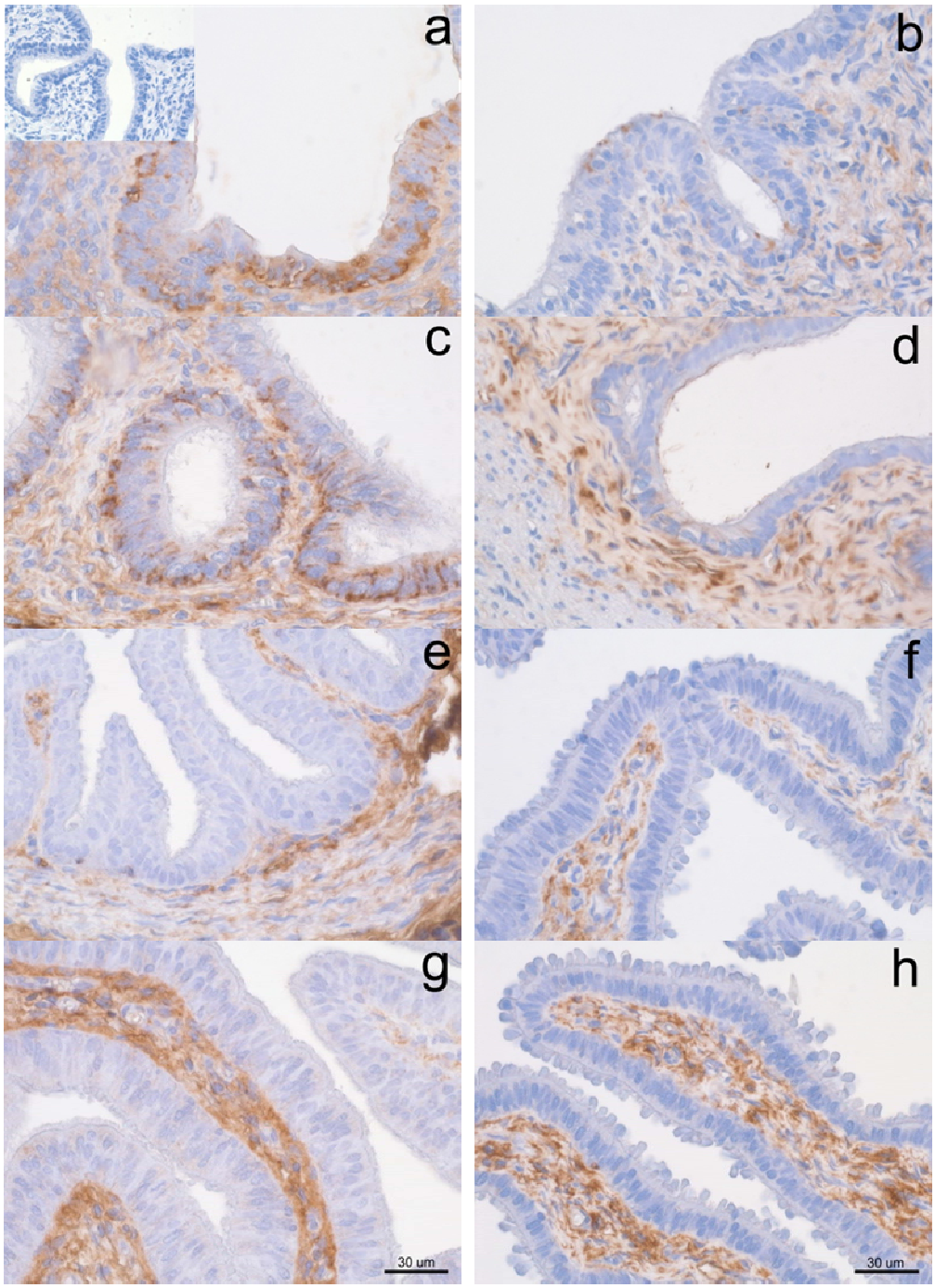


**Figure 3** Immunohistochemical localization for syndecan-1 in the sections of the UTJ (a, b), isthmus (c, d), ampulla (e, f), and infundibulum (g, h) of the swamp buffalo oviducts at the follicular (a, c, e, g) and mid-luteal (b, d, f, h) phases. Inset in the UTJ (a) depicts the negative control. Bars = 30 µm.

***Syndecan-1 immunolocalization:*** In the positive control sections (Fig 2) the uterine epithelium (a) and the epithelium of endometrial glands (b) demonstrated strong positive staining with syndecan-1, whereas the control sections did not demonstrate any staining definitely (inset in Fig 3a). In the buffalo oviducts, syndecan-1 was frequently seen along the epithelial lining of all oviductal segments (Fig 3a-h). The strong positive staining of syndecan-1 was definitely found in the epithelium whereas the weak staining might possibly be noticed in the subepithelial connective tissue compartment. The pattern of syndecan-1 immunolabeling in the epithelium demonstrated that the cytoplasmic and apical surface staining of the oviductal epithelial cells was present at follicular and mid-luteal phases. It is noticed that the apical surface of syndecan-1 staining remarkably appeared in the UTJ (Fig 3a and 3c) and isthmus (Fig 3b and 3d) at both phases. However, the syndecan-1 labeling in the cytoplasm was observed in the epithelial cells of ampulla (Fig 3e and 3g) and infundibulum (Fig 3e & 3f) but not all epithelial cells of both segments were seen.

## *Discussion*

The present study confirmed that the non-sulphated GAGs or HA and one example of heparan sulphated GAGs, i.e. syndecan-1 were localized in the swamp buffalo oviductal tissues. Importantly, HA was predominantly found in the UTJ and isthmus, the site of sperm storage, during the follicular phase which was the critical time for sperm preservation before ovulation. In the mean time, syndecan-1 obviously appeared along the lining epithelium of all segments at both estrous cycle stages. However, the intensity of syndecan-1 staining appearing on the apical epithelial surface was remarkably observed at the UTJ and isthmus. Detailed information in our research might be important to realize the contribution of intraluminal oviductal secretions and gametes during the normal fertilization process in the swamp buffalo oviduct.

It is documented that the basic knowledge involved in the physiological regulations of female swamp buffalo reproductive organs has not been studied well whereas this category including reproductive biotechnology in female cows has greatly been progressive (Madan, 2005; Perera, 2008). We have known that the sperm reservoir in farm animals supplies several functions such as the preservation of sperm fertilizing proficiency to expand the time between sperm deposition and ovulation, the procedure of sperm capacitation and the modulation of sperm transportation to the position of fertilization or preventing of polyspermy (Hunter, 2005). To complete the fertilization processes, the spermatozoa must pass on the UTJ and adjacent isthmus, region of sperm reservoir, where it certainly appears to have a particular responsibility for those spermatozoa to survive (Topfer-Petersen et al., 2002). Normally, spermatozoa stored in a sperm reservoir were normally entrapped within the epithelial crypts by binding to the epithelial cells or the secretions present on the apical epithelial surface (Hunter et al., 1991) and these substances in the luminal fluid prompt the release of viable spermatozoa to the upper segment at peri-ovulation time by the events of capacitation (Talevi and Gualtieri, 2010). In the present study, both HA and syndecan-1 could be found in the swamp buffalo oviduct, particularly in the sperm reservoir, in accordance with previous studies in pigs (Tienthai et al., 2000) and cows (Bergqvist et al., 2005a, Bergqvist and Rodriguez-Martinez, 2006). Considering the non-sulphated GAGs, the HA is a main composition within the extracellular matrix, particularly in the loose connective tissue (Laurent et al., 1995). Therefore, the appearance of HA in the subepithelial connective tissue or lamina propria in all segments of swamp buffalo oviduct was regularly found. However, the remarkable question is where the HA in the swamp buffalo oviductal epithelial cells and also epithelial surface, especially in the sperm reservoir (UTJ and isthmus) at the follicular phase, comes from. With the present result, it is possible that HA found in the lining epithelium may be synthesized by the epithelial cells in the sperm reservoir as reported in the porcine (Tienthai et al., 2003) and bovine (Ulbrich et al., 2004) oviducts. It is accepted that HA is synthesized by hyaluronan synthesizing enzymes or hyaluronan synthases (HAS) which are composed of HAS-1, HAS-2 and HAS-3 (Spicer et al., 1996). Morover, the earlier studies suggested that HAS-2 and HAS-3 genes were detected in the bovine oviduct (Ulbrich et al., 2004), whereas only HAS-3 gene was expressed in the sperm reservoir of the porcine oviduct (Tienthai et al., 2003). The types of HAS genes, however, in the swamp buffalo oviduct need to be investigated in future studies.

In our previous study, Tienthai et al. (2004) reported that most of the spermatozoa collected from the sow sperm reservoir (UTJ), where they contained with HA, were still viable and shown uncapacitated status. Moreover, the ultrastructure of the sperms in the UTJ contacted with epithelial cells or their secretions showed intact plasma membrane during pre-ovulation as well (Mburu et al., 1997). Furthermore, the additional exposure with HA in these findings approved that HA via intraluminal fluid localized in the sperm reservoir was capable to maintain sperm viability and could be involved in arresting sperm capacitation during the critical period of follicular phase. In the bovine, a lot of experiments investigating the effects of the exposure of HA on the sperm status *in vitro* suggested that the HA was able to induce some proportion of spermatozoa to be capacitated without causing an increase in acrosome reaction in bull spermatozoa (Lee and Ax, 1984; Januskaukas et al., 2000; Bergqvist et al., 2006). Therefore, HA might serve as the ordinary sperm selector in the cow oviduct and is a very useful medium containing in the swim-up process to prepare spermatozoa during *in vitro* fertilization (Shamsuddin et al., 1993). Moreover, various biological functions of HA need to be completed by interacting through the

specific transmembrane receptor, i.e. CD44 (Alho and Underhill, 1989) because the CD44 was detected in the oviductal epithelium of porcine (Tienthai et al., 2003)

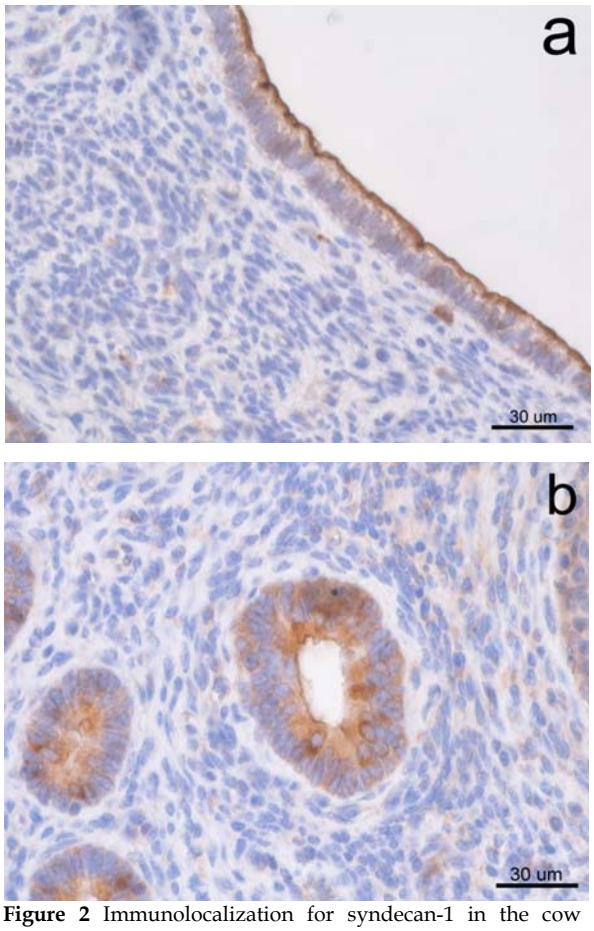


**Figure 2** Immunolocalization for syndecan-1 in the cow uterine horn section was served as the positive control slides. The brown staining was clearly found in the uterine epithelial lining (a) and also found in the epithelium of endometrial glands. Bars = 30 μm.

and bovine (Ulbrich et al., 2004; Bergqvist et al., 2005[b]). The CD44 participates in various cellular functions, e.g. cell differentiation, cell-cell adhesion, cell-matrix adhesion, uptake and degradation of HA, inhibition of apoptosis and immune response (Underhill, 1992; Culty et al., 1992). Importantly, HA-CD44 pathways have appeared to be associated with cell signaling (Turley et al., 2002). With the variety of functions suggested above, it can be stated that HA in the buffalo sperm reservoir might particularly be associated with attachment of spermatozoa and epithelial cells. Although the exact mechanism of HA, which related to the maintenance of sperm viability or fertilizing ability within sperm reservoir, was unclear. The localization of CD44 in the swamp buffalo oviduct and the influence of the additional exposure of HA on buffalo spermatozoa demand for further notice.

The immunolocalization of syndecan-1 in the swamp buffalo oviduct of this study was intensely found in the surface epithelium and also in the cytoplasm of epithelial cells throughout estrous cycle corresponding to earlier studies in sows (Tienthai et al., 2000) and cows (Bergqvist and Rodriguez-Martinez, 2006). In the present study, we used anti-human syndecan-1 because it is suppose to react similarly in all species since the amino acid sequence of the cytoplasmic tails of syndecan-1 of animals and humans are identical (Pierce et al., 1992). Absolutely, important mechanism in the oviduct that researchers are interested in the sperm capacitation, which is an alteration of the biochemical procedures in membrane destabilization of spermatozoa before reaching acrosome reaction during fertilization time (Yanagimachi, 1994). Among the variety of capacitation stimulators, heparin is a useful substance for promoting capacitation in bull spermatozoa *in vitro* (Parrish et al., 1988, 1989). Although there is no report yet about the presence of heparin-like GAGs in the female reproductive tract of swamp buffalo, heparin has also been applied in *in vitro* fertilization (IVF) for decades (Madan et al., 1994; Chauhan et al., 1997). However, the achievement rate of IVF with heparin-stimulated buffalo spermatozoa is pretty deprived comparable to cows (Nandi et al., 2006). Interestingly, the recent study by Roy and Atreja (2009) indicated that the event of sperm capacitation relating to the protein tyrosine phosphorylation pattern in the buffalo spermatozoa occurred very early as compared to cows. The basic structure of heparin and heparan sulphate is almost similar, but heparan sulphate contains fewer sulphate groups and fewer iduronic acid units compared to heparin (Kjellen and Lindahl, 1991). Possibly, the presence of heparan sulphate in the pattern of syndecans within the swamp buffalo oviduct might be associated with the capacitation processes or other mechanisms of buffalo spermatozoa in the different conditions.

We have known that the syndecans are transmembrane heparan sulphate proteoglycans which are composed of syndecan-1 to syndecan-4 and interestingly syndecan-1 is the main type of syndecan that is expressed in the epithelial cells (Zimmermann and David, 1999) as shown in this research. However, various function of syndecan-1 has been implicated in the mechanisms of cell differentiation, proliferation, adhesion and migration via cytoskeleton and microfilament reorganization (Carey et al., 1994a,b). Recently, Zong et al. (2011) has studied the regulation of syndecan-1 in mesenchymal tumor cells and they suggested that syndecan-1 decreased the migration and motility, but enhanced adhesion of mesenchymal tumor cells in a level-dependent manners. As mentioned above, the sperm reservoir served as the place to maintain the sperm viability and initiate sperm capacitation before releasing the spermatozoa to the site of fertilization at ovulation time. Therefore, syndecans located in the swamp buffalo sperm reservoir might join with HA to increase sperm-epithelial cell adhesion during follicular phase. At luteal phase, the concentration of syndecan-1 in the oviductal tissues and fluids decreased (Tienthai et al., 2000; Bergqvist and Rodriguez-Martinez, 2006) indicating that it is possible that syndecan-1 during this phase might increase cell motility and migration of spermatozoa.

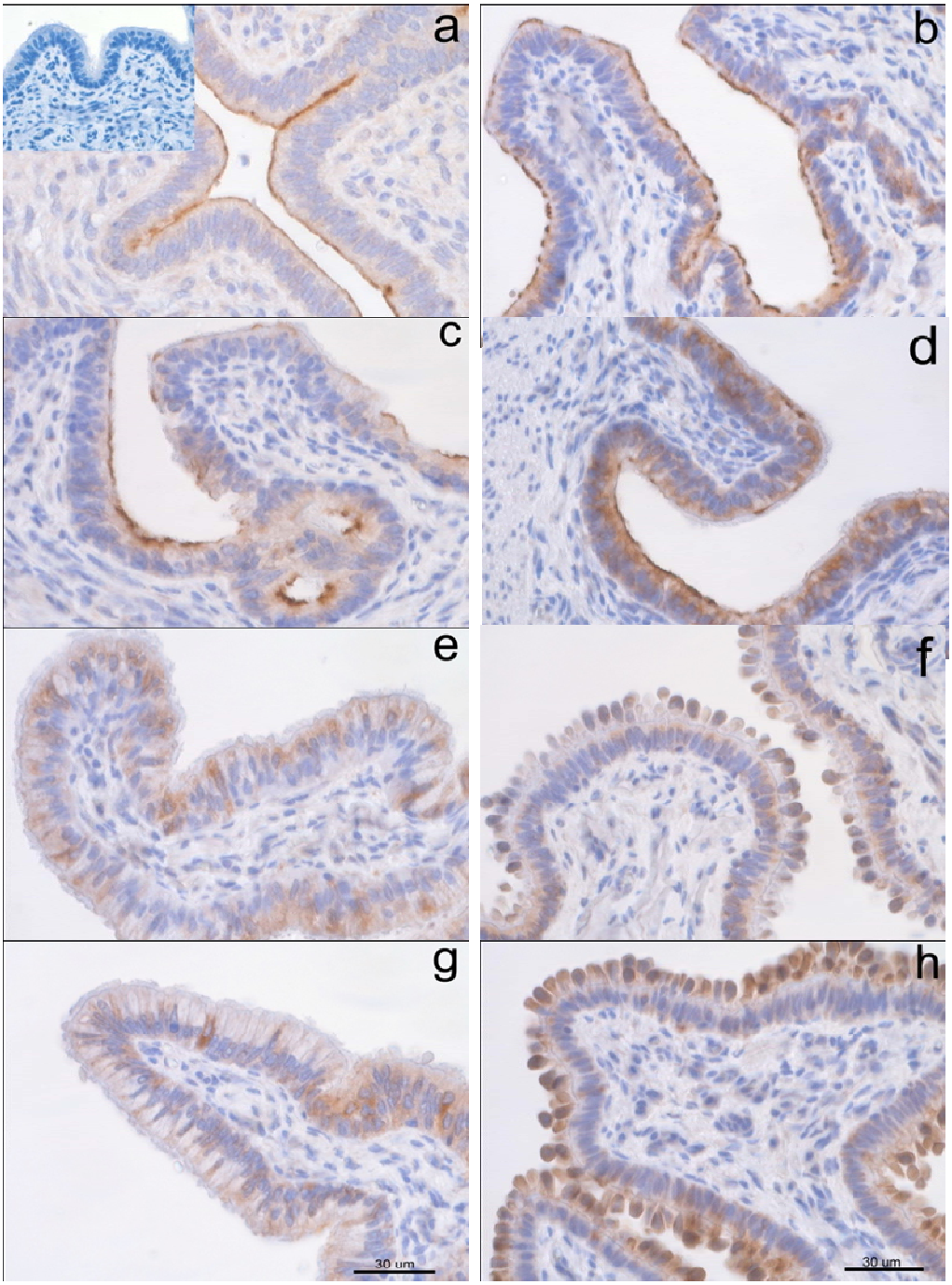


**Figure 3** Immunohistochemical localization for syndecan-1 in the sections of the UTJ (a, b), isthmus (c, d), ampulla (e, f), and infundibulum (g, h) of the swamp buffalo oviducts at the follicular (a, c, e, g) and mid-luteal (b, d, f, h) phases. Inset in the UTJ (a) depicts the negative control. Bars = 30 µm.

## *Conclusion*

In conclusion, the present study indicates for the first time that both non-sulphate GAGs or HA and sulphated GAGs in the pattern of heparan sulphated proteoglycans which signified syndecan-1 were localized in the swamp buffalo oviductal epithelium, principally in the UTJ and isthmus or the sperm reservoir. The presence of HA promotes the sperm adhesion and the maintenance of sperm survival while syndecan-1 might function with HA to increase sperm adhesion and then it may initiate the sperm capacitation and regulate sperm motility during luteal phase. However, the HA receptors and the HA synthases in the swamp buffalo oviduct including the mechanisms of oviductal fluid and the individual type of GAGs on the buffalo sperm status by flow cytometry procedures remain to be explored in future studies.

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