Epithelial Cadherin Immunolocalization in the Sperm Reservoir of Thai Swamp Buffalo

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Introduction

The swamp buffalo population in Thailand is dramatically decreased by numerous reasons. The factors for this animal are composed of delayed puberty, long calving interval and poor detection of estrus including reproductive disturbances, e.g. early embryonic death and reduced conception rates (1). These causes have been the main restrictions on improved productivity in swamp buffalo. To increase the production, an essential understanding with mention to the molecular biology in the female reproductive organs, particularly in the oviduct must be investigated. In cattle, the oviducts play a vital role before fertilization, transporting the oocyte from through the infundibulum and ampulla to the place of fertilization (2). The caudal isthmus and uterotubal junction (UTJ), so called “sperm reservoir”, are associated in procedures such as sperm transport, storage and capacitation that require preservation of the motility, viability and fertilizing ability of spermatozoa (3). Recently, the hyaluronan and syndecan-1 were definitely contained in the sperm reservoir of swamp buffalo and these substances could involve in the forming of sperm reservoir to maintain sperm viability (4). However, the accurate modulation during sperm-epithelium adhesion within sperm reservoir is still obscured and requires further information. Cadherins are a family of transmembrane glycoproteins that motivate calcium-dependent cell adhesion and they are recognized according to their tissue of derivation composed of epithelial (E), neural (N) and placental (P) cadherins (5). E-cadherin was the first cadherin identified to play an essential role in mediating selective adhesion between epithelial cells and involved in the initial attachment of the embryo to the endometrium (6). E-cadherin was detected in bovine oviduct epithelium, oocyte and spermatozoa (7). Thus, the purpose of this research was to detect E-cadherin in the UTJ and isthmus of Thai swamp buffalo by immuno-histochemistry.

Materials and Methods

The female reproductive organs from swamp buffaloes (n=20) were obtained at the local abattoirs. The chosen stages of estrous cycle, the follicular (n=10) and mid-luteal phases (n=10), were sorted by the manifestation of corpus luteum and dominant follicle on both ovaries (8). The oviducts were separated from mesosalpinx and then the uterotubal junction (UTJ) and caudal isthmus were collected and submerged in 10% buffered formalin. The oviduct tissues in paraffin blocks were cut into 4-μm-thick sections and placed on the Poly-L-Lysine coated glass slides (Sigma-Aldrich, Steinheim, Germany), deparaffinized in xylene and rehydrated through graded ethanol dilutions. Thereafter the sections were put in 0.01 M citrate buffer (pH 6.0) in a microwave oven at 750 W. Endogenous peroxidase activity was inhibited by immersing the sections in 3.0% H_2O_2 at room temperature and a non-specific background staining was reduced by incubation with normal horse serum (Vector Laboratories, Burlingame, CA, USA). Mouse monoclonal antibody to E-cadherin (clone NCH-38, DAKO, Grostrup, Denmark) at a dilution of 1:50 was served as primary antibody. Subsequently, the sections were applied with the secondary biotinylated horse anti-mouse antibody (Vector Laboratories, Burlingame) at a dilution of 1:200 followed by Avidin-Biotin Complex (ABC)-mouse reagent (Vector Laboratories). The positive reactions were visualized using freshly prepared the 3, 3'-diaminobenzidine (DAB kit, Vector laboratories) in H_2O_2 and all sections were counterstained with Mayer's hematoxylin and mounted with gelatin-glycerine mixture. All tissue sections were evaluated under light microscopy (BX50, Olympus, Tokyo, Japan) with a digital camera Micropublisher 5.0 (Qimage, Surrey, Canada). The tissue micrographs were taken by program of Image Pro® Plus version 6 (Media Cybernatics Inc., MD, USA).

Results and Discussion

As expected, the strong intensity of E-cadherin staining was detected in canine mammary gland carcinoma which served as the positive controls (Fig. 1a, b), whereas no staining was present on the negative controls (inset panel in Fig. 1a). The pattern of E-cadherin expression was clearly observed at the lateral and apical membranes in cells of mammary gland carcinoma (Fig. 1b).

The E-cadherin immunohistochemical reaction was depicted with strong intensity at the

cell-to-cell borders of the epithelial linings of UTJ and caudal isthmus both follicular and mid-luteal phases, whereas a weak immunostaining was appeared within the cytoplasm of these epithelial linings in various area (Fig. 2).

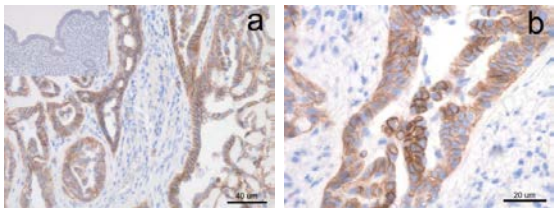

Figure 1 Intense immunolocalization of E-cadherin (dark brown stain) in canine mammary adenocarcinoma cells (positive control) at different magnifications (a, b) that principally demonstrated the membranous E-cadherin staining pattern (b). No positive immunohistochemical staining was detected in negative controls (inset panel).

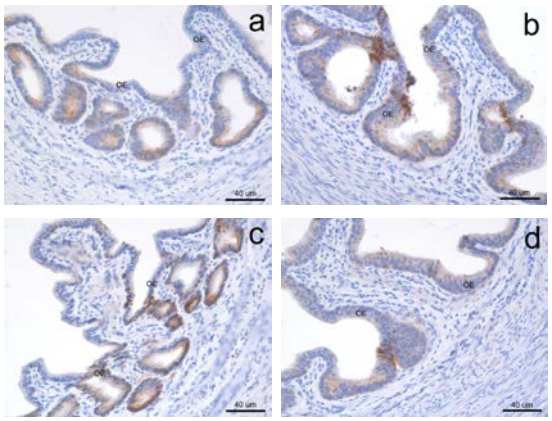

Figure 2 Immunohistochemical staining of E-cadherin in the UTJ (a, b) and caudal isthmus (c, d) at follicular (a, c) and mid-luteal (b, d) phases. Strong E-cadherin appearance was found at the lateral and apical cell membranes of oviduct epithelium (OE) and the positive staining was intermittently depicted throughout the epithelium.

In cattle, it is known that UTJ and caudal isthmus serve as sperm reservoir where supplies numerous functions, e.g. the maintenance of sperm fertilizing proficiency to expand the time during sperm storage until ovulation, the procedure of sperm capacitation and the modulation of sperm transportation to the site of fertilization (3, 9). To comprehensive the complex fertilization processes, the spermatozoa have to store within sperm reservoir to have accountability for spermatozoa to survive and to maintain more capability (10). Recently, glycosaminoglycans (both hyaluronan and syndecans) were found in the swamp buffalo oviduct epithelium of the UTJ and isthmus and these substances might benefit the maintenance of sperm survival (11).

Among members of cell-cell adhesion proteins, the presence of E-cadherin in bovine gametes and oviduct epithelium supporting their role in gamete interaction (7). By experimental design, the reallocate in E-cadherin localization was also scrutinize in spermatozoa released from co-cultures, indicating the involvement of the adhesion protein in assembly or disassembly of the oviduct-sperm reservoir, as part of the capacitation-related events (12). Previous studies implied the involvement of species-specific carbohydrate recognition in sperm-oviduct interaction and specific proteins have been advised to play a vital role in the formation of the sperm reservoir (9, 13, 14). Various kinds of proteins appear to be lost from the plasma membrane overlying the head during sperm capacitation, and this event is related to a decrease in oviduct epithelium-sperm binding (15). Therefore, E-cadherin could be component of this complicated array of membrane proteins participating in this incident to assure sperm association and/or release from the oviduct epithelium of sperm reservoir.

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References

1. Singh et al., 2000. Anim Reprod Sci. 60-61: 693-604.
2. Ellington, 1991. Cornell Vet. 81: 313-328.
3. Pollard et al., 1991. Biol Reprod. 44: 102-107.
4. Tienthai, 2011. Thai J Vet Med. 41: 327-335.
5. Tsuchiya et al., 2006. Arch Histol Cytol. 69: 135-145.
6. Dawood et al., 1998. Am J Obstet Gynecol. 178: 996-1001.
7. Caballero et al., 2014. Theriogenology. 81: 1189-1206.
8. Chandra Roy et al., 2006. Theriogenology. 65: 1292-1301.
9. Hunter, 2005. Reprod Nutri Dev. 45: 281-290.
10. Topfer-Petersen et al., 2002. J Exp Zool. 292: 210-215.
11. Tienthai, 2011. Thai J Vet Med. 41: 327-335.
12. Pollard et al., 1991. Biol Reprod. 44:102-117.
13. Gwathmey et al., 2003. Biol Reprod. 69: 809-815.
14. Suarez, 2001. Cells Tissues Organs. 168: 105-112.
15. Tollner et al., 2008. Biol Reprod. 78: 400-412.