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Relationship between Plasminogen Activity and Plasminogen Inhibitor during the Culture of Porcine Oviduct Epithelial Cells

Shin-Hye Ahn[1], Hee-Tae Cheong[2], Boo-Keun Yang[1], Dae-Young Kim[3] and Choon-Keun Park[1,†]

[1]College of Animal Life Science and [2]School of Veterinary Medicine, Kangwon National University, Chuncheon 200-701, Korea
[3]Division Biological Science, Gacheon University of Medical and Science, Incheon 406-799, Korea

ABSTRACT

The present study was performed to identify changes of plasminogen activator (PA) and plasminogen activator inhibitor (PAI) in porcine oviduct epithelial cells (POECs) during the estrous cycle. POECs obtained from ovary in pre-ovulatory (Pre-Ov), early to mid-luteal stage (Early-mid L) and post-ovulatory stage (Post-Ov). For the examine of PA activity, 1×10^5 fresh cells of POECs were cultured in DMEM/Ham F-12 containing 10% FBS and 0.2% amphotericin under humidified atmosphere of 5% CO_2 in air and 38℃. The urokinase-type PA (uPA) was observed at 7 days of POECs culture. PA activity was measured with culture prolonged of 0, 3, 6, 12 and 24 h after culture of 7 days. The PA activity were high significantly ($p<0.05$) at 12 h of culture, but PA activity were decreased with culture periods increased. The PA activity in POECs of Post-Ov stage were higher significantly ($p<0.05$) than that of Early-mid L and Pre-Ov stage. When PAI-1 and PAI-2 were added during the POECs culture, the PA were observed significant low activity ($p<0.05$). The PA activity and protein expression were decreased by PA inhibitor. This results suggest that PAI-1 and PAI-2 have a suppressive action on change of PA activity during the estrous cycle of pigs. Specifically, this study using PA inhibitor was effect the PA activity and PAI expression in oviduct epithelial cells in pigs.

(Key words : Plasminogen activator, Oviduct epithelial cells, Estrous cycle, PA-inhibitor, Pig)

INTRODUCTION

Plasminogen activator (PA) are specific serine proteolytic enzymes that convert the inactive proenzyme plasminogen to plasmin. The plasmin formed is a non-specific potent protease that cleaves blood fibrin clots and several other extracellular protein (Dano *et al.*, 1985). Also, cell surface associated plasmin catalyzes the breakdown of the extracellular matrix and basement membrane molecules, such as fibronectin, laminin (Liotta *et al.*, 1981), vitronectin (Chain *et al.*, 1991), proteoglycans (Mochan and Keler, 1984), fibrin (Dudek *et al.*, 1970) and collagen (Liotta *et al.*, 1981; Vassalli *et al.*, 1991), (Mochan and Keler, 1984). Plasminogen activator/plasmin can exert directly and indirectly extracellular matrix degradation (Smokovitis *et al.*, 1989). Several observations suggest that the PA/plasmin system might also have a role in mammalian oocyte fertilization, sperm motility and the acrosome reaction (Taitzoglou *et al.*, 2003, 2004). Although these PAs are believed to be important in extracellular tissue remodeling in many physiological process, including fibrinolysis, ovulation, involution, implantation and pathological process including cardiovascular diseases and tumor metastasis (Schmitt *et al.*, 1997). The unrestranined generation of plasmin from plasminogen by the action of PA is potentially hazardous to cells (Irigoyen *et al.*, 1999). Plasminogen activators (PAs), which convert plasminogen into the active form plasmin. Two types of PA have been described the urokinase-type (uPA), and the tissue-type (tPA). Plasminogen activation is involved in many cellular functions that require proteolytic degradation of extracellular matrix (Andreasen *et al.*, 2000). The activity of PA is regulated by specific plasminogen activator inhibitor (PAI-1 and PAI-2) (Lecander *et al.*, 1984; Loskutoff *et al.*, 1989). Plasminogen activator inhibitor-1 (PAI-1) is a 50 kDa protein belonging to a family of serine protease inhibitors known as serine PAI-1 is synthesized by a variety of cells and is induced by growth factors, cytokines, hormones, and other stimuli, and high PAI-1 levels are present in plasma from patients with acute or chronic inflammatory conditions. PAI are member of the serine protease inhi-

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† Corresponding author : Phone: +82-33-250-8627, E-mail: parkck@kangwon.ac.kr

bitor and inhibitor include PAI-1 and PAI-2 is a single chain 45～50 kDa glycoprotein secreted by many cell types. The secreted form is quickly transformed into a dormant conformation unable to interacting with PAI-1 can stabilize the active conformation and broaden the substrate form complexes with PAs. The physiological cofactors vitronectin and heparin, by specificity toward a further serine protease thrombin. Besides the direct PA-inactivating role, PAI-1 binding to the ECM also modulates pericellular proteolysis carried out by receptor bound uPA. PAI-1 is also involved in the regulation of cell adhesion and migration. PAI-2 is a single-chain protein of 47 kDa, and it's inhibitory potency measured as an association constant towards receptor bound uPA is 15 times less than that of PAI-1 (Ellis *et al.*, 1990). The main function of PAI-1 in tissue is to inhibit the action of the urokinase type plasminogen activator (uPA), a serine protease involved in tissue remodeling and cell migration. The regulatory proteases participate in the extensive disruption of cell and extra cellular matrix (ECM) contacts. PAI-1 is one of the prototypic molecules for analyzing stimulatory effects of POECs on PAI-1 protein expression. As a member of the serine protease family PAI-1 regulates the plasminogen system by inhibiting two plasminogen activators (PA), urokinase PA (uPA) or tissue-type PA (tPA). The interaction between uPA and PAI-1 and PAI-2 in the porcine oviduct estrous cycle is not very well understood either. Theses molecules may play important roles during PA formation, function, and regression in the primate. The aim of the present study was carried out to evaluate PA in the Pre-Ovulatory, Early to mid-luteal and Post-Ovulatory stages of the porcine oviducts in different stages of the estrous cycle.

MATERIALS AND METHODS

Collection and Culture of Porcine Oviduct Epithelial Cells (POECs)

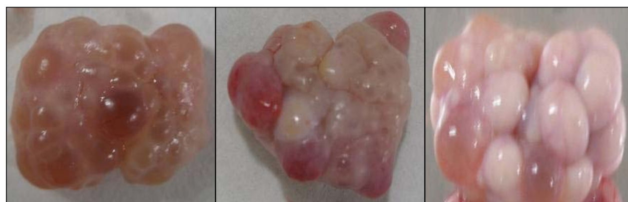

Fig. 1. A photography of ovaries during the estrous cycle in pig. The estrous cycles were distinguished by diameter of follicles and corpora lutea of ovary. The POECs were isolated from oviduct of ovary classified the by estrous cycles.

Porcine oviducts during the estrous cycle were collected at a local slaughterhouse and kept in Hank's balanced salt solution (HBSS) at 4℃. The stage of the estrous cycle was defined by careful examination of the ovaries. The classification of the oviducts into three groups were the following (Fig. 1).

Cell populations were separated using a modification of procedure previously described (Zhang *et al.*, 1999). The oviduct was washed 3 times with 30～50 ml of sterile Ca^{2+} and Mg^{2+} free Hank's balanced salt solution (HBSS) supplemented with 100 IU/ml penicilin G, 100 μg/ml streptomycin and 0.1% (w/v) BSA (Roche Diagnosties GmbH, Mannheim, Germany). 30～45 ml of sterile HBSS containing 0.3% (w/v) trypcin (Sigma Chemical Co., St. Louis, MO; #T4665) was then infused into the uterine lumen through the catheter. The filtrate was washed 3 times by centrifugation (1,500 rpm, 5 min) with Dulbecco's Modified Eagle's medium (DMEM; sigma; # D1152) and Phosphate buffer saline (PBS) supplemented with antibiotics and 0.1% BSA. After the washing the cells were counted with a hematocytometer. The cell viability was higher than 85% as assessed by 0.5% (w/v) trypan blue dye exclusion. The final pellet of both the epithelial cell were separately resupended in culture medium (DMEM/ Ham's F-12; 1:1 (v/v): Sigma; #8900) supplemented with 10%. Dulbecco's Modified Eagle's Medium Nutrient Mixture F-12 Ham supplemented with 10% FBS and 0.2% amphotericin B plated in 6 well culture dishes, 1×10^5 cell/ml were cultured at 5% CO_2 in air at 38℃ for 7 days. The medium was changed every 2 days until the cell was confluenced.

PA Activity Assays

Total PA activity levels in the medium were measured using the chromogenic assay as described originally by Hart and Rehemtulla (1988) with some modifications. Briefly samples were incubated with or without (nagative control) 2.5 mg of plasminogen (Sigma, # P5661) 96-well microtiter plates at 37℃ for 60 min to generate plasmin, Chromogenic substrated for plasmin, Z-L-Lys-SBzl hydrochloride (0.18 mM; Sigma; #C3647) and 5,5′-dithio-bis (2-nitrobenzoic acid: 0.22 mM; Sigma #C8130) were then added, and the incubation was continued at 37℃ for another 60min. The absorbance at 405 nm was determined using an automated microplate reader. Standard curves were constructed using 0～190ng/ml of human urokinase (ProSpec-Tany Techno Gene; ENZ-264). No PA activity was detected in the absorbance of plasminogen (negative control).

Western Blot Analysis

The primary antibodies were used with goat anti-uPA (Santa Cruz Biotechnology) and goat anti-PAI-1 (SantaCruz Biotechnology) for rabbit anti-PAI-2 (Santa

Crua) and mouse anti-actin (Santa Cruz Biotechnology).

Secondary horseradish peroxidase-conjugated antibodies were obtained from chemicon and stressgen respectively. Sample containing 40 μg protein were separated by SDS-PAGE consisting of 5% (w/v) acrylamide stacking gel and a 15% (w/v) separating gel containing 0.1% SDS. The running buffer was composed of 0.1% SDS, 25 mM Tris and 250 mM glycine (pH 8.3). Electrophoretic fractionation was carried out at a constant current of 25 mA. Protein were then electroblotted onto a nitrocellulose membrane. The filters were blocked with 5% skim milk in PBS containing 0.1% Tween 20 for incubation. at RT with the primary antibody specific to each protein (uPA, PAI-1, PAI-2 and β-actin). After washing in TBST, the filter were incubated 1 h at RT with the respective HRP-conjugated anti-immunoglobulin (1:10,000). Following three washes in TBST bands were visualized with enhanced chemiluminescence reagent and subsequent exposure to hyperfilm enhanced chemiluminescence.

Flow Cytometry and Attractor Analysis

Analyses were performed on modified FACS count flow cytometers with a fiber coupled 488 nm external laser (Beckman coulter: cytomics™ FC 500). This instrument collects single parameters of fluorescence and on parameter of size data for each event. uPA and PAI-1 secondary antibody used Alexa Fluor® 488 (A-11034) and PAI-2 secondary antibody used Alexa Fluor® 546 (A11010). uPA and PAI-1 secondary antibody approximate fluorescence emission (Em) 519 and excitation (Ex) 495 emission wavelength and PAI-2 secondary antibody approximate fluorescence emission (Em) 573 and excitation (Ex) was 556. All experiment was progressed 4℃, confluence cells were collect and fixed in 70% ethanol for 30 min. After centrifuge at 1,500 rpm for 5 min and the cells washed in PBS, and were suspended at 2×10^6 prior to acquisition. The cells added with uPA, PAI-1 and PAI-2 were preserved in shaking in 70% ethanol for 1 h and washed with PBS. Second fluorescence antibody was mixed by vortexing for 5 sec and RT, and subjected to flow cytometric analysis.

Immunofluorescent Staining and Confocal Microscopy

Being stained, cells were fixed with PBS for 30 minutes on ice, washed, and absorbed to cover slips. For the confocal microscopy, cells were fixed at the confluence cell incubated directly with 0.5 μg/ml of purified antibody that had been diluted in 1 ml of PBS for 1 hour. Cells were then resuspended with 1 ml PBS solution that contained an uPA, PAI-1 and PAI-2 (Santa Cruz). uPA and PAI-1 were followed by incubation with Alexa Fluor 488 donkey anti-goat immunoglobulin G (H+L) antibody (Invitrogen) that was diluted 1:10,000 in 1 ml PBS 1% for 60 minutes and PAI-2 were Alexa Fluor 546 goat anti-rabbit immunoglobulin G (H+L) antibody (Invitrogen). Cells were embedded in mounting medium and analyzed with the confocal microscopy (Zeiss LSM 510, Jena Germany).

Statistical Analysis

The data were analyzed by the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS Institute Inc., Car, NC). PA activity and expression in each treatment were compared for differences through use of Duncan's modified multiple range test. Data from each experiment are presented as the means ± S.E.M. and $p<0.05$ were considered to be statistically significant.

RESULTS

The first experiments were examined effects of the during the estrous cycle on plasminogen activator activity in porcine oviduct epithelial cells. As a results, PA activity significantly higher ($p<0.05$) in Post-Ov stage during the estrous cycle. When PA activity were measured at 0, 3, 6, 12 and 24 h after more culture of confluenced cells, PA activity was higher in Post-Ov than Early to mid-luteal stage and Pre-Ov stages at 12h of POECs culture (Fig. 2).

To confirm of uPA, PAI-1 and PAI-2 protein expression in cultured porcine oviduct epithelial cells, this study were focused on the porcine estrous cycle for determination of PA activity. As shown in Fig. 3 and 4(A). the uPA protein expression were significantly ($p<0.05$) higher in Post-Ov and Early to mid-L stages than that of the Pre-Ov stage. However, PAI-1 protein expression (B in Fig. 4) were significantly ($p<0.05$) high-

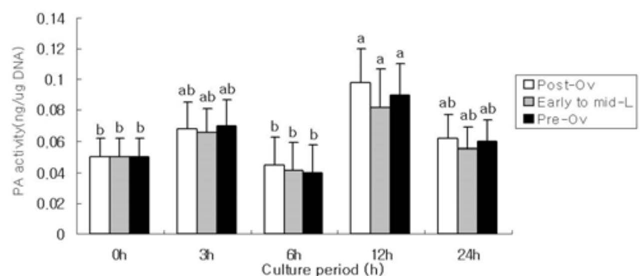

Fig. 2. Effects of estrous cycle on plasminogen activator activity in porcine oviduct epithelial cells. Plasminogen activator activity expressed in PA of tissue in the three segments of the porcine oviduct during the estrous cycle. Value are means and standard deviations of optical density from triplicate independent experiment, [a~c] Different superscripts are significantly different ($p<0.05$).

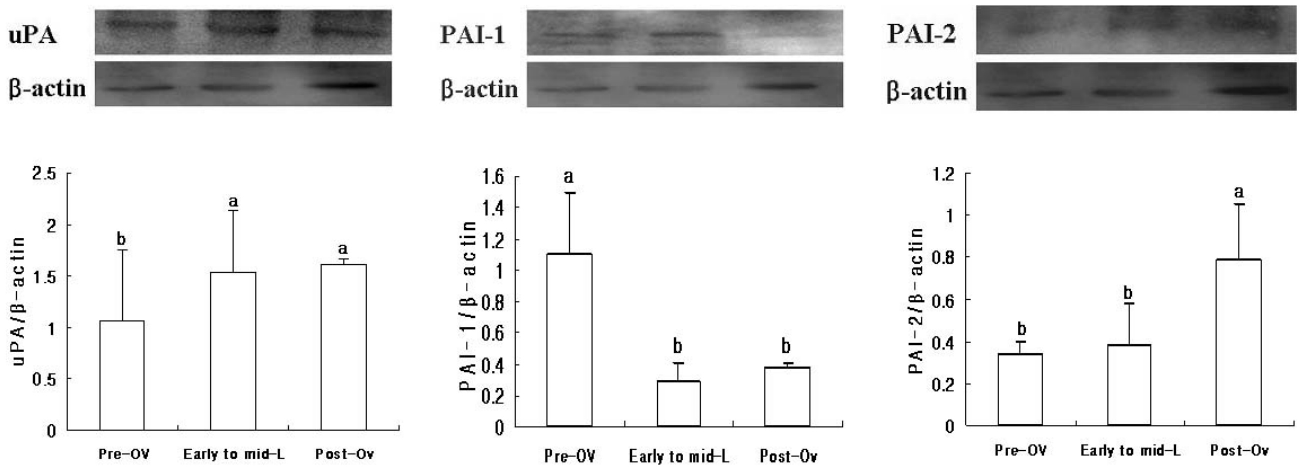

Fig. 3. Regulation of uPA, PAI-1 and PAI-2 induced PA protein expression in porcine oviduct epithelial cells. Expression regulated by different pharmacological agents. Value are means and standard deviations of optical density from triplicate independent experiment. [a~c] Different superscripts are significantly different (p<0.05).

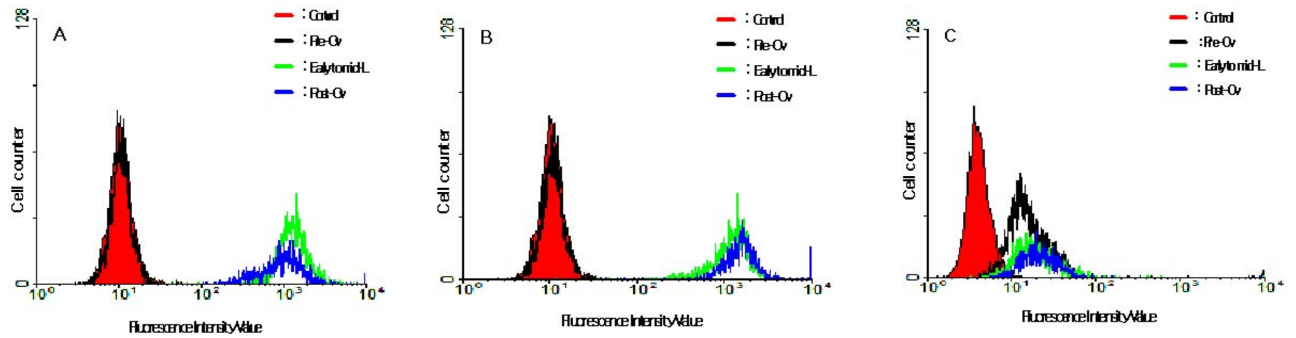

Fig. 4. Flowcytometric histogram showing fluorescence signals, generated by porcine oviduct epithelial cells with A) uPA, B) PAI-1 and C) PAI-2 for protein expression (p<0.05).

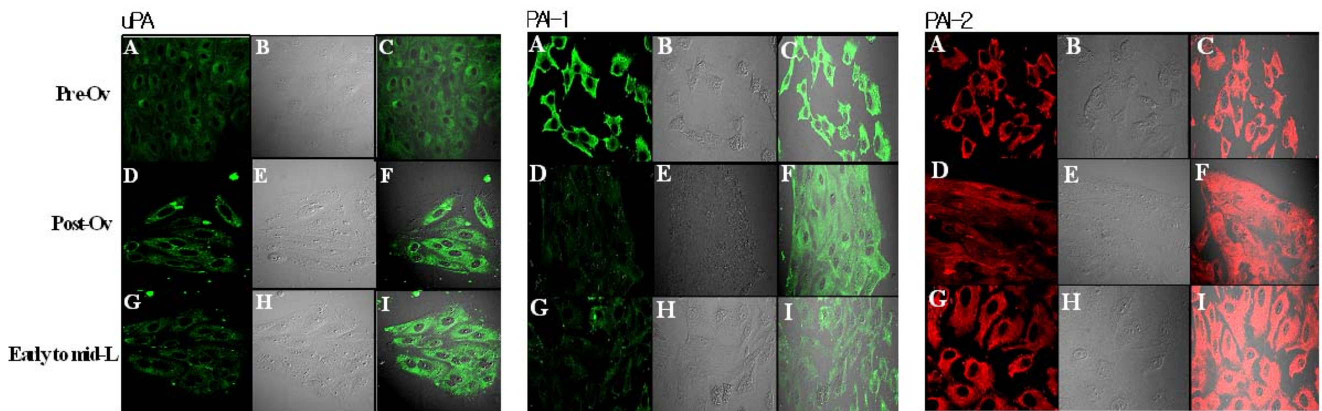

Fig. 5. Fluorescent imaging by confocal laser scanning microscope in porcine oviduct epithelial cells. POECs: A, B, C; Pre-Ov stage, D, E, F; Post-Ov stage, G, H, I; Early to mid-L Image: A, D, G; by bright microscope, B, E, H; by general microscope, G, H, I; by overlay.

er Pre-Ov stage than Early to mid-L and Post-Ov stages.

On the other hand, PAI-2 protein expression (C in Fig. 4) was significantly (p<0.05) higher Pre-Ov stage

than Post-Ov and Early to mid-L stages.

Fig. 5 showed confocal image by FITC after uPA, PAI-1 and PAI-2 protein expression in POCEs. Fluorescence showed strongly Post-Ov and Early to mid-L stages than Pre-Ov stage, these results demonstrated uPA protein expression in Fig. 3 and 4. On the other hand, Fluorescence of Pre-Ov stage showed strongly than Post-Ov and Early to mid-L stages on PAI-1 protein expression of POECs. However, fluorescence intensity of POECs expressed with PAI-2 protein was not difference among the estrous cycle.

DISCUSSION

This present study was undertaken to identity changes of the PAs activity by culture period dependent of oviduct epithelial cells during the estrous cycle in pig. The mammalian oviduct secretes into the oviductal lumen that influence gamete interaction, fertilization process and early cleavage stages of embryonic development the action of these proteins differ with oviductal region as well as with stages of estrous cycle. It is possible that PA may be involved in these processes in a specific and finely regulated proteolytic cascade. It has been reported that PA release into the porcine oviduct epithelial cells may contain the PA receptor to bind uPA to the cell surface. It has been found that plasminogen dependent protease in POECs conditioned medium of different stage of the estrous cycle. In this study, PA activity was observed during the culture of POECs in estrous cycle. Specially, PA activity was significantly higher in the POECs conditioned medium of Post-Ov stage (Fig. 2). The participation of uPA, PAI-1 and PAI-2 in the mechanism of fertilization or early embryo development has not been studied yet, it is possible that uPA, PAI-1 and PAI-2 maybe involved in these processes in a specific and finely regulated proteolytic cascade. Hart and Rehemtulla (1988) reported that uPA is secreted as an inactive single chain molecule of 31～54 kDa. In this study uPA, PAI-1 and PAI-2 activity was detected, PAI-2 protein expression was higher than that of PAI-1 in all stage of estrous cycle. The present study demonstrated that uPA was activated in the porcine oviduct through uPA activity in POECs conditioned medium. In this study, uPA, PAI-1 and PAI-2 protein expression were confirmed in POECs of Post-Ov, Early to mid-L and Pre-Ov stage. This variation appeared to be constant regardless stages of the estrous cycle. The uPA activity was significantly (p<0.05) different during the culture of POECs of estrous cycle. Maybe porcine oviduct epithelial cells contain the uPA receptor to bind uPA to the cell surface in porcine oviduct epithelial cells. Extracellular proteolysis linked with plasminogen activator (PA)/plasmin system activity, is believed to be involved in physiological processes such as cumulus cell expansion and dispersion (Canipari *et al.*, 1995), fertilization (Rekkas *et al.*, 1993), zona reaction (Lee and Wei, 1994) and embryo implantation (Bray *et al.*, 1975). In this study, the highest plasminogen activator activity was measured in the Post-Ov stage, and decreased during the Pre-Ov stage. This discrepancy is probably due to the overwhelming presence of PA inhibitor in the Pre-Ov. It is well known that PAI-1 and PAI-2 the main inhibitor or plasminogen activator/plasmin system is present in the porcine oviductal fluid as well as in the apical region of the epithelial cells, greater amount of PAI-1, PAI-2 protein expression was detected in the Pre-Ov regardless of the estrous cycle. Oviductal epithelium, protein composition of oviductal fluids and oviductal fluid volume vary according to the hormonal status of the female and appear to be controlled by ovarian estrous and progesterone (Lippes and Wagh, 1993; Mastroianni and Go, 1979; Verhage and Jaffe, 1986). Also, the synthesis and release of plasminogen activator and plasminogen activator inhibitor are modulated in several cell types by hormone (Kokolis, 1990; Schatz and Tsantarliotou, 1997). Reports on PAI-1 and PAI-2 regulation in endometrial stromal and decidual cell cultures suggest that this protein is up regulated by progesterone, while estrogen antagonizes this effect (Kokolis, 1990). The oviduct along with the uterus, is a major target for ovarian steroids. It's suggest the changes of PAs activity by steroid hormone that the present work demonstrate that the changes of activity of plasminogen acitivators at the different stages of the estrous cycle in porcine oviduct epithelial cells. This study supports the concept that urokinase-type plasminogen activator a component of extracellular matrix, is produced and secreted by the porcine oviduct in stable manner during the estrous cycle. The PA activity in the oviductal lumen is variable due to inhibitory control and the mechanism probably under influence of ovarian steroids. This study observed that expression of uPA, PAI-1 and PAI-2 during the estrous cycle. It is postulated that PAI plays a role in the modulation of activity function, including the enhancement of PA activity. Therefore, this study demonstrate that the changes of actvitiy of plasminogen activators at the different stages of the estrous cycle in porcine oviduct epithelial cells, and it's suggest that may be play various contral role of PAs according to the estrous cycle in oviduct of pig.

REFERENCES

1. Andreasen PA, Egelund R, Petersen HH (2000): The plasminogen activatrion system in tumor growth, invasion, and metastasis. Cell Mol Life Sci 57:25-40. Review.
2. Bray BA, Hsu KC, Wigger HJ, LeRoy EC (1975): Association of fibrinogen and microfibrils with trophoblast basement membrane. Connective Tissue Research 3:55-71.
3. Canipari R, Epifano O, Siracusa G, Salustri A (1995): Mouse oocytes inhibit plasminogen activator production by ovarian cumulus and granulosa cells. Developmental Biology 167:371-378.
4. Chain D, Kreizman T, Shapira H, Shaltiel S (1991): Plasmin cleavage of vitronectin: identification of the site and consequent attenuation in binding plasminogen activator inhibitor-1. FEBS Lett 285:251-256.
5. Dano K, Andersen PA, Grondahl-Hansen J, Kristensen P, Nilsen LS, Skriver J (1985): Plasminogen activators, tissue degradation and cancer. Adv Cancer Res 44:139-266.
6. Declerck PJ, De Mol M, Alessi MC, Baudner S, Paques EP, Preissner KT, Muller-Berghaus G, Collen D (1988): Purification and characterization of a plaminogen activator inhibitor 1 binding protein from human plasma. Identification as a multimeric form of S protein (vitronectin). Journal of Biological Chemistry 263:15454-15461.
7. Dudek GA, Kloczewiak M, Budzynski AZ, Latallo, ZS, Kopec M (1970): Characterisation and comparison of macromolecular end products of fibrinogen and fibrin proteolysis by plasmin. biochim. Biochim Biophys Acta 214:44-51.
8. Ellis V, Wun TC, Behrendt N, Ronne E, Dano K (1990): Inhibition of receptor-bound urokinase by plasminogen-activator inhibitors. J Biol Chem 265: 9904-9908.
9. Hart DA, Rehemtulla A (1988): Plasminogen activators and their inhibitors: regulators of extracellular proteolysis and cell function. Comparative biochemistry and physiology B Comparative Biochemistry 90:691-708.
10. Irigoyen JP, Oz-Cao Anoves P, Montero L, Koziczak M, Nagamine Y (1999): The plasminogen activator system: Biology and regulation. Cellular and Molecular Life Science 56:104-132.
11. Kokolis NA (1990): Effect of orchidectomy on tissue plasminogen activator activity, plasminogen activator inhibition and plasmin inhibition. Haemostasis 20:233-240.
12. Lecander I, Roblin R, Astedt B (1984): Differential inhibition of two molecular forms of melanoma cell plasminogen activator by a placental inhibitor. Br J Haematol 57:407-412.
13. Liotta LA, Goldfarb RH, Brundage R, Siegal GP, Terranova V, Garbisa S (1981): Effect of plasminogen activator (urokinase), plasmin and thrombin on glycoprotein and collagenous components of basement membrane. Cancer Res 41:4629-4636.
14. Lippes J, Wagh PV (1993): Human oviductal fluid proteins. VI. Correlation between alpha-fetoprotein and serum levels of ovarian hormones. Fertility and Sterility 59:157-162.
15. Loskutoff DJ, Sawdey M (1989): Type 1 plasminogen activator. Progress in Hemostasis and Thrombosis 9:87-115.
16. Mastroianni L, Go KJ (1979): The Biology of the Fluids of the Female Genital Tract. Elsevier/North Holland pp 335-344.
17. Mochan E, Keler T (1984): Plasmin degradation of cartilage proteoglycan. Biophys Acta 800:312-315.
18. Lee SL, Wei YH (1994): The involvement of extracellular proteinases and proteinase inhibitors in mammalian fertilization. Biotechnology and Applied Biochemistry 19:31-40.
19. Rekkas C, Kokolis N, Smokovitis A (1993): Breed and seasonal variation of plasminogen activator activity and plasminogen activator inhibition in spermatozoa and seminal plasma of the ram in correlation with testosterone in the blood. Andrologia 25:101-109.
20. Schatz F, Lockwood CJ (1993): Progestin regulation of plasminogen activator inhivitor type 1 in primary cultures of endometrial stromal and decidual cells. Journal of Clinical Endocrinology and Metabolism 77:621-625.
21. Schmitt M, Harbeck N, Thomssen C, Wilhelm O, Magdolen V, Reuning U, Ulm K, Fler H, Nicke F, Graeff H (1997): Clinical impact of the plasminogen activation system in tumor invasion an metastasis: Prognostic relevance and target for therapy. Thrombosis and Haemostasis 78:285-296.
22. Smokovitis A, Kouimtzis S, Koutsouris C, Kokolis N, Kouskoura T (1989): The effects of intrafollicular injection of plasmin and ε-aminocaproic acid on the ovulation in the ewe. Fibrinolysis 3:227-230.
23. Taitzoglou IA, Chapman DA, Killian GJ (2003) : Induction of the acrosome reaction in bull spermatozoa with plasmin. Andrologia 35:112-116.
24. Taitzoglou IA, Chapman DA, Zervos IA, Killian GJ (2004): Effect of plasmin on movement characteristics of ejaculated bull spermatozoa. Theriogenology 62:553-561.
25. Tsantarliotou M (1997): The effect of Glucocorticoids and Melatonin on the Activity of Specialized Proteases in Spermatozoa and Seminal Plasma of the Ram. Thessaloniki.
26. Vassalli JD, Sappino AP, Belin D (1991): The plasminogen activator/plasmin system. J Clin Invest 88:1067-1072.

27. Verhage HG, Jaffe RC (1986): Regulation of estradiol and progesterone receptor concentration in cat uteri following chronic progesterone administration. Journal of Steroid Biochem 24:587-590.
28. Zhang A, Paria BC, Davis DL (1999). Pig endometrial cells in primary culture: morphology, secretion of prostaglandins and proteins, and effects of pregnancy. J Anim Sci 69:3005-3015.

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