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Effect of estrogen on prostaglandin synthetase in bovine oviduct smooth muscle

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ABSTRACT

Gamete and embryo transport is an important function of the oviduct. This type of transport involves both smooth muscle contraction and epithelial cell secretions, and the former is mediated by prostaglandins (PGs) and their receptors. Our objective was to study the regulation of prostaglandin synthetase (prostaglandin-endoperoxide synthase-1 (PTGS1), prostaglandin-endoperoxide synthase-2 (PTGS2), mPGES-1, mPGES-2, cPGES, and PGFS) by estradiol (E_2) in bovine oviduct smooth muscle. Prostaglandin synthetase mRNA and protein expression were investigated using real-time RT-PCR and Western blot analyses, respectively. Prostaglandin synthetase mRNA and protein expression were increased in oviductal smooth muscle tissue after treatment with different concentrations of estradiol for various time periods. The results indicated that there was no increase in expression observed after treatment with fulvestrant, a selective antagonist of the E_2 receptor, indicating that E_2 interacts with specific E_2 nuclear receptors to upregulate PTGS1, PTGS2, mPGES-1, and PGFS expression. In conclusion, E_2 increases PTGS1, mPGES-1, and PGFS mRNA and protein expression in bovine oviductal smooth muscle when added for different periods of time and at different concentrations. Additionally, E_2 is transported intracellularly and interacts with specific E_2 nuclear receptors to increase PTGS1, PTGS2, mPGES-1 and PGFS expression.

1. Introduction

The oviduct, the site where an oocyte and sperm join to form first a zygote and then a primary embryo, is an important organ that affects reproductive capacity (Acosta et al., 1998). Fertilization must be precisely initiated and regulated, and the transport and storage of sperm and ovum depend on a functioning oviduct, which also provides an optimal internal environment for zygote development.

PG synthesis occurs through a complex process in which phospholipase A_2 catalyzes membrane phospholipids to release arachidonic acid (AA). AA is then converted to prostaglandin H_2 (PGH_2) through the action of prostaglandin-endoperoxide synthase-1 (PTGS1) or prostaglandin-endoperoxide synthase-2 (PTGS2) (Smith and Song, 2002), and PGH_2 is subsequently transformed into distinct PGs by isomerases (Narumiya and FitzGerald, 2001), e.g., to PGE_2 by prostaglandin E synthases (PGES) and to $PGF_{2\alpha}$ by prostaglandin F synthases (PGFS). Three PGES subtypes exist: microsomal prostaglandin E synthase-1 (mPGES-1), microsomal prostaglandin E synthase-2 (mPGES-2), and cytosolic prostaglandin E synthase (cPGES). A particular cell type may predominantly express one or more than one isomerase, which largely determine the prostaglandin generated (Ueno et al., 2005).

The transport of spermatozoa is likely aided by muscular contractions in the oviduct wall (Mastroianni, 1999), and muscular activity is regulated by adrenergic nerves, sex steroids (Helm et al., 1982; Sjoberg, 1967), and PGs (Caschetto et al., 1979; Lindblom et al., 1983). Key reproductive events are strictly controlled by estrogen and progesterone (Acosta et al., 1998). Sex steroids may also modulate PG receptor expression (Blesson et al., 2012). In cattle, estradiol (E_2) administration, as early as day 13 of the estrous cycle, causes an increase in the plasma concentration of 13,14-dihydro-15-ketoprostaglandin $F_{2\alpha}$ (PGFM), the main metabolite of $PGF_{2\alpha}$ (Knickerbocker et al., 1986; Thatcher et al., 1986).

Rosenkrans reported that estrogens in the endometrium increase the ratio of PGE_2 to $PGF_{2\alpha}$ and may be important for the establishment of

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pregnancy (Rosenkrans et al., 1990). Indeed, the balance between these two molecules affects much of the female reproductive system, including the ovary, uterus, and oviduct. In the oviduct, PGE_2 and $PGF_{2\alpha}$ control both the secretory activities of oviductal epithelial cells to promote a suitable fertilization environment and oviductal contraction for optimal embryo transport, whereas an imbalance in the $PGE_2/PGF_{2\alpha}$ ratio causes pathological effects that can lead to infertility. These findings suggest that the participation of PGs in this process is essential for the development of new life; however, the distinct association between estrogen and PGs, particularly in the oviduct, are largely unknown.

The objective of the present study was to determine the time-effect relationship of estrogen on the prostaglandin synthetase expression in bovine oviductal smooth muscle.

2. Materials and methods

2.1. Ethics statement

All animal studies were conducted in accordance with the experimental practices and standards approved by the Animal Welfare and Research Ethics Committee at Inner Mongolia Agricultural University (Approval ID:20130927-1), and all efforts were made to minimize animal suffering.

2.2. Tissue culture

Fresh oviducts with intact ovaries from 60 sexually mature crossbred heifers (approximately 6 months old, non-pregnant) were provided by a local abattoir and used in the experiments. The heifers were determined to be in proestrus by gross examination of the follicle size (smaller than 2 mm in diameter) and by the appearance of corpora lutea. The oviductal smooth muscle segment in the ampulla (approximately 15 mm in length) was carefully surgically isolated from the utero-ovarian ligament, the mesosalpinx, and other extraneous tissues. The oviduct was then cut longitudinally, and the endosalpinx was removed using fine scissors. Muscle preparations were suspended vertically in a culture bottle (50 mL) containing Dulbecco's modified Eagle's medium (DMEM)/F12 (no phenol red, HyClon, Logan, Utah, America), dextran-coated charcoal-treated fetal bovine serum (FBS; 15 mL/100 mL), and mycillin (1 mL/100 mL). The muscle strip was loaded at an initial tension of 0.5 g, incubated for 7 days, and bubbled with 5% CO_2 in a constant-temperature incubator at 37 °C to maintain the physiological characteristics of bovine oviduct smooth muscle and eliminate endogenous E_2 interference (Huang et al., 2015). The remaining smooth muscle preparation was used for real-time PCR and Western blot analyses.

2.3. Immunohistochemistry

The freshly obtained oviduct ampulla regions were fixed for 48 h in 4% formaldehyde, frozen, cut into 5-μm-thick sections, and then treated with H_2O_2 to block endogenous peroxidase. The samples were then blocked with 10% goat serum and incubated with polyclonal primary antibodies against PTGS1 (Cayman Chemical Company, MI, USA) diluted 1:100, PTGS2 (Cayman Chemical Company, MI, USA) and mPGES-1 (Cayman Chemical Company, MI, USA) diluted 1:100, mPGES-2 (Cayman Chemical Company, MI, USA) diluted 1:50, cPGES (Cayman Chemical Company, MI, USA) diluted 1:50, PGFS C1orf93 antibody (Abcam, Cambridge, England) diluted 1:50 at 4 °C overnight, followed by incubation with goat anti-rabbit or secondary antibody. Next, the samples were subjected to DAB for visualization. Finally, the samples were counterstained with haematoxylin. In negative controls, rabbit IgG was substituted for primary antibodies. The sections were then counterstained with haematoxylin, which was followed by dehydration and mounting with Pertex. A Olympus microscope was used to evaluate the slides.

2.4. Treatment

2.4.1. Effect of E_2 treatment duration

Bovine oviductal smooth muscle tissues (n = 28) were divided into seven groups, and four independent representative experiments were conducted for each group. Six groups were treated with 10^{-11} mol/L E_2 for 2, 4, 8, 16, 24, or 48 h. The control group was treated with FBS alone. Based on its physiological concentration in bovines, estrogen was used at 10^{-11} mol/L (Huang et al., 2015; Kesler et al., 1976; Mason et al., 1972).

2.4.2. E_2 mechanism

Bovine oviductal smooth muscle tissues (n = 24) were divided into four groups, and six independent representative experiments were conducted for each group. According to the results of 2.4.1, five groups were treated with 10^{-11} mol/L E_2 for 8 h, 10^{-6} mol/L fulvestrant for 4 h, 10^{-6} mol/L fulvestrant for 1 h + 10^{-11} mol/L of E_2 for 8 h, 10^{-5} mol/L tamoxifen for 4 h, 10^{-5} mol/L tamoxifen for 1 h + 10^{-11} mol/L of E_2 for 8 h. The control group was treated with FBS alone.

2.5. RNA extraction, reverse transcription and real-time PCR

Total RNA was extracted using RNAiso Plus (TaKaRa DRR9109) according to the manufacturer's instructions. cDNA was prepared using a PrimeScript™ RT reagent kit (TaKaRa RR037A). Real-time PCR was performed using SYBR Premix Ex Taq™ II (TaKaRa RR820A). cDNA was amplified (Vii A7; Applied Biosystems) using the following conditions: initial denaturation for 30 s at 95 °C followed by 40 cycles of

Table 1
Primers used in this study.

Genes (bp)	Accession no.	Nucleotide sequence (5′ – 3′)	Length
PTGS1	NM_001105323.1	Forward 5′GCCATCCGAACTCCATC3′ Reverse 5′CCCACATCACCACCAAAT3′	229 bp
PTGS2	NM_174445.2	Forward 5′CGAGGTGTATGTATGAGTGTAG3′ Reverse 5′TTTGAAGTGGGTAAGTATGTAG3′	302 bp
mPGES-1	NM_174443.2	Forward 5′CGCTGCTGGTCATCAAAAT3′ Reverse 5′GGAAGGGGTAGATGGTCTCC3′	186 bp
mPGES-2	NM_001166554.1	Forward 5′ATCAGCAAGCGGCTCAAACG3′ Reverse 5′TCTCAGCGTCGCCAAGTGC3′	368 bp
cPGES	BC103350.1	Forward 5′AAGGAGAATCTGGCCAGTCA3′ Reverse 5′ATCCTCATCACCACCATGT3′	170 bp
PGFS	D88749.1	Forward 5′GATGGCCACTTCATTCCTGT3′ Reverse 5′CACAGTGCCATCTGCAATCT3′	195 bp
β-actin	NM_173979.3	Forward 5′CCAAGGCCAACCGTGAGAAGAT3′ Reverse 5′CCACGTTCCGTGAGGATCTTCA3′	256 bp