Anim. Reprod., Belo Horizonte, v.12, n.2, p.328-335, Apr./Jun. 2015

Expression of epidermal growth factor (EGF) and its receptor in bovine endometrium throughout the luteal phase: effects of EGF on prostaglandin production in endometrial cells

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Abstract

Epidermal growth factor (EGF) is produced in bovine endometrium throughout the estrous cycle. However, little is known about the expression of the EGF receptor (EGFR) and the roles of EGF in bovine endometrium. To clarify whether EGF is involved in local regulation of bovine endometrial function, first we determined the EGF protein and the expression of *EGFR* mRNA in endometrial tissues throughout the luteal stages. EGF protein concentration was higher ($P < 0.05$) in the mid (days 8-12) luteal stage than in the other luteal stages. *EGFR* mRNA expression was higher ($P < 0.05$) in the mid and late (days 15-17) luteal stages than in the other luteal stages. To investigate the protein concentrations of EGF and *EGFR* mRNA expression in cultured bovine endometrial cells, epithelial and stromal cells were isolated between day 0 and day 4 post-ovulation from 22 uteri. Both EGF protein concentration and *EGFR* mRNA expression were higher ($P < 0.05$) in epithelial cells than in stromal cells. Then, to examine the possible role of EGF in the regulation of prostaglandin F2α (PGF2α) and prostaglandin E2 (PGE2), cultured endometrial epithelial and stromal cells were exposed to EGF (0, 1, 10 and 100 nm) for 24 h. In epithelial cells, EGF (10 and/or 100 nm) increased ($P < 0.05$) PGF2α and PGE2 secretion, but in stromal cells EGF (100 nm) increased ($P < 0.05$) PGF2α, but not PGE2 secretion. These results indicate that 1) the highest amount of EGF is produced by bovine endometrium at the mid-luteal stage, 2) endometrial *EGFR* mRNA expressions are higher at mid and late-luteal stages than other stages, 3) EGF is expressed mainly by uterine epithelial cells and 4) EGF has the ability to increase PGE2 and PGF2α production in both epithelial and stromal cells and therefore may play a role in local regulation of uterine function.

Keywords: bovine endometrium, epidermal growth factor, prostaglandins.

Introduction

Epidermal growth factor (EGF) stimulates cell growth, proliferation and differentiation in numerous tissues by binding to its receptor (EGFR). EGF is a polypeptide composed of 53 amino acids with a mitogenic effect (Akbalik and Ketani, 2013) that is expressed in the endometrium of cattle (Katagiri and Takahashi, 2004), sheep (Gharib-Hamrouche *et al*., 1995), goats (Tamada *et al*., 2000) and pigs (Kennedy *et al*., 1994). Several observations suggest that EGF and EGFR help maintain fertility and normal uterine function in cattle (Katagiri and Takahashi, 2004, 2006, 2008). Uterine concentrations of EGF in repeat breeder cows on day 3 and day 14 of the estrous cycle are lower than those of fertile cows, which conceived after the first or second artificial insemination (Katagiri and Takahashi, 2004). Furthermore, immunohistochemical studies in bovine endometrium revealed that EGFR expression was greater in the mid-luteal stage than in the regressed luteal stage, and that EGFR displayed stronger immunoreactions in luminal and superficial glandular epithelial cells than in basal glandular epithelial and stroma cells (Sağsöz *et al*., 2012).

Prostaglandins (PGs) are bioactive lipids produced from arachidonic acid that is converted to prostaglandin E2 (PGE2) and prostaglandin F2α (PGF2α) by specific enzymes (Arosh *et al*., 2002). In cows, the pulsatile release of PGF2α from the uterus after day 17 of the estrous cycle is known to induce luteolysis (Okuda *et al*., 2002), whereas PGE2 has been suggested to be a luteoprotective factor (Weems *et al*., 2006). PGs produced by bovine endometrial epithelial and stromal cells help to control the expression of implantation-related genes around the time of implantation (Dorniak *et al*., 2011). In rat, intraluminal infusion of PGF2α increased the number of implantation sites (Oettel *et al*., 1979). In mice, both PGE2 and PGF2α induce morphological changes in trophoblastic cells and increase the implantation rate (Holmes and Gordashko, 1980). These observations suggest that PGE2 and PGF2α regulate not only luteolysis but also implantation. The effects of EGF on PGE2 and PGF2α production in bovine endometrial cells are poorly understood.

No information is available concerning the cell-specific expression of EGFR and EGF protein in bovine endometrium. We hypothesized that EGF is involved in regulating bovine endometrial function by

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Received: October 17, 2014
Accepted: February 6, 2015

regulating prostaglandin production locally. To test the above hypothesis, we determined 1) the concentration of EGF protein and the expression of *EGFR* mRNA in endometrial tissues throughout the luteal stages, 2) the protein concentrations of EGF and *EGFR* mRNA expression, and 3) the effects of EGF on the production of PGE2 and PGF2α in endometrial epithelial and stromal cells.

Materials and Methods

Collection of endometrial tissues

Uteri of Holstein cows were obtained from a local abattoir according to protocols approved by the local institutional care and use committee. Uteri were obtained within 10-20 min after exsanguinations and were immediately transported to the laboratory. Luteal stages were confirmed by macroscopic observation of the ovary and uterus as described previously (Miyamoto *et al*., 2000). For mRNA (n = 5 cows/stage) and protein (n = 3 cows/stage) determination, endometrial tissues were collected from cows at five different luteal stages (early: days 2-3; developing: days 4-6; mid: days 8-12; late: days 15-17 and regressed luteal stages: days 19-21). Intercaruncular endometrial tissues from the uterine horn, ipsilateral to the CL, were used for experiments. Endometrial tissues were immediately frozen in liquid nitrogen, and stored at -80°C until processed for mRNA extraction and protein isolation.

Isolation of endometrial cells

The epithelial and stromal cells from the bovine endometrium were isolated between day 0 and day 4 post-ovulation from 22 cows using a modified procedure previously described (Murakami *et al*., 2003). A polyvinyl catheter was inserted into the side of the oviduct, and the ends of the horn were tied to retain trypsin solution for detaching the epithelial cells as described below. The uterine lumen was washed three times with 30-50 ml of sterile Ca^{2+} free and Mg^{2+} free Hanks balanced salt solution (HBSS) supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin, and 0.1% (w/v) bovine serum albumin (BSA; Roche Diagnostics, Manheim, Germany; 10735086001). Thirty to fifty milliliters of sterile HBSS containing 0.3 (w/v) trypsin (Sigma-Aldrich Chemical Co, St. Louis, MO, USA #T4665), deoxyribonuclease I [Sigma-Aldrich; #D5025], and 0.1% BSA) was then infused into the uterine lumen through the catheter. Epithelial cells were isolated by incubation at 37°C for 30 min with gentle shaking.

After collection of the epithelial cells, the uterine lumen was washed with sterile HBSS supplemented with antibiotics and 0.1% (w/v) BSA. The horn was then cut transversely using scissors into several segments, which were slit to expose the endometrial surface. Intercaruncular endometrial strips were dissected from the myometrial layer with a scalpel and washed once in 50 ml of sterile HBSS containing antibiotics. The endometrial strips were then minced into small pieces (1 mm^3). The minced tissues (5 g) were digested by stirring for 45 min in 50 ml sterile HBSS containing 0.05% (w/v) collagenase (Sigma-Aldrich; #CO130), 0.005% (w/v) deoxyribonuclease I and 1% BSA. The dissociated cells were filtered through metal meshes (100 and 80 µm) with Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich; #D1152) supplemented with antibiotics and 0.1% BSA. The filtrates were washed by centrifugation at 180 x g for 10 min with Tris-buffered ammonium chloride (pH 7.5) to remove hemocytes, and then washed twice with DMEM supplemented with antibiotics and 0.1% BSA. After the washes the cells were counted with a hemocytometer. The cell viability was higher than 85% as assessed by 0.5% (w/v) trypan blue dye exclusion.

Culture of endometrial cells

The pellets of the stromal and epithelial cells were separately resuspended in culture medium (DMEM/Ham's F-12; 1:1 (v/v); Sigma-Aldrich; #D8900) supplemented with 10% (v/v) calf serum (Sigma-Aldrich; #C6278), 20 µg/ml gentamicin (Invitrogen Co., Carlsbad, CA; #15750-060) and 2 µg/ml amphotericin B (Sigma; #A9528). These cells were seeded at a density of 1×10^5 viable cells/ml in culture flasks (Greiner Bio-One, Frickenhausen, Germany; #662160) and cultured at 38.0°C in a humidified atmosphere of 5% CO2 in air. To purify the stromal preparation the medium was changed 2 h after seeding, by which time selective attachment of stromal cells had occurred (Fortier *et al*., 1988; Skarzynski *et al*., 2000). Since the epithelial cells attached 24-48 h after plating, the medium in the epithelial cell culture was replaced 48 h after seeding. The medium was changed every 2 days until the cells reached confluence. The homogeneity of stromal and epithelial cells was evaluated using immunofluorescent staining for specific markers of epithelial (cytokeratin) and stromal cells (vimentin) as described previously (Malayer and Woods, 1998; Tanikawa *et al*., 2008). When cells of each type were confluent (6-7 days after the start of the culture), the cells were cultured for another 24 h in fresh DMEM/Ham's F-12 medium supplemented with 0.1% BSA, 5 ng/ml sodium selenite (Sigma-Aldrich; #S5261), 0.5 mM ascorbic acid (Wako Pure Chemical Industries, Ltd., Osaka, Japan; #013-12061), 5 µg/ml transferrin (Sigma-Aldrich; #T3400), 2 µg/ml insulin (Sigma-Aldrich; #I4011), and 20 µg/ml gentamicin. Cultured endometrial epithelial (n = 4 cows) and stromal (n = 4 cows) cells were incubated with EGF (0, 1, 10 and 100 nm; Teikoku Hormone MFG Co., Tokyo, Japan) in fresh DMEM/Ham's F-12 medium supplemented with 0.1% BSA. After 24 h incubation the

culture media were collected for determination of PGE2 and PGF2α concentration. For total RNA extraction cells were washed twice with PBS and digested by addition of TRIsure (Bioline, London, UK; #BIO-38033), and then stored at -80°C until use. For protein analysis the cells were scraped and placed in ice-cold homogenization buffer (25 mM Tris-HCl, 300 mm sucrose, 2 mM EDTA, Complete [protease inhibitor cocktail; Roche Diagnostics; 697498], pH 7.4), frozen in liquid nitrogen and then stored at -80°C until EGF protein analysis by enzyme-linked immunosorbent assay (ELISA).

Total RNA extraction and quantitative RT-PCR

Total RNA was extracted from endometrial tissue using TRIsure (Bioline, London, UK; #BIO-38033) according to the manufacturer's directions. Extracted RNA from each sample was quantified using a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) with absorbance of light (260 nm). One microgram of total RNA was reverse transcribed using a ThermoScript RT-PCR System (Invitrogen), and 10% of the reaction mixture was used in PCR using specific primers for *EGFR* and *GAPDH* for the bovine sequence. The primers were chosen using Primer3, an online software package (http://primer3.sourceforge.net/). *GAPDH* expression was used as an internal control. The sequences of the *EGFR* primers, 5'-AGTTGGGCACTTTTGAAGACC-3' (5' primer, 21 mer) and 5'-AGGACCACCTCACAGTTGTTG-3' (3' primer, 21 mer), were synthesized according to bovine *EGFR* cDNA (Gen Bank accession number XM_002696890.2), and these primers generated a specific 67-base pair (bp) product from all cell types. The primers for *GAPDH* were 5'-CACCCTCAAGATTGTCAGCA-3' (5' primer, 20 mer) and 5'-GGTCATAAGTCCCTCCACGA-3' (3' primer, 20 mer). These primers generated a specific 103-base pair (bp) product from all cell types (GenBank accession number NM_001034034.2). For quantification of the mRNA expression levels, the primer length (20-21 bp) and guanine and cytosine content of each primer (45-55%) were selected. The protocol conditions consisted of denaturation at 95°C for 30 s, followed by 40 cycles at 95°C for 5 s, 64°C for 10 s with a final dissociation (melting) curve analysis. The use of the iQ SYBR Green supermix at elevated temperatures resulted in reliable and sensitive quantification of the RT-PCR products with high linearity (Pearson correlation coefficient r > 0.99). To analyze the relative level of expression of each mRNA, the 2-ΔΔCT method was used (Livak and Schmittgen, 2001).

Hormone determination

The concentrations of EGF in the collected endometrial tissues and cultured cells were determined by bovine epithelial growth factor (EGF) ELISA Kit (#CSB-E17171B, Cusabio Biotech Co., Ltd. Hubei, China). Endometrial tissues and cultured cells were homogenized on ice in the homogenization buffer by a tissue homogenizer (Physcotron; Microtec Do., Chiba, Japan; NS-50), followed by filtration with a metal wire mesh (150 μm). For EGF protein analysis nuclei were isolated from the tissue homogenates by centrifugation at 600 x g for 30 min. Protein concentrations in the lysates were determined using the method of Osnes *et al*. (1993) with BSA as a standard. The EGF standard curve ranged from 0.156 to 10 ng/ml, and the ED50 of the assay was 1.25 ng/ml. The intra-assay coefficients of variation were on average 4.0%.

The concentrations of PGE2 and PGF2α in the collected cultured media were determined by enzyme immunoassay as described previously (Uenoyama *et al*., 1997; Tanikawa *et al*., 2005). The PGE2 standard curve ranged from 0.039 to 10 ng/ml, and the ED50 of the assay was 0.625 ng/ml. The intra- and inter-assay coefficients of variation were on average 2.7 and 9.5%, respectively. The PGF2α standard curve ranged from 0.016 to 4 ng/ml, and the ED50 of the assay was 0.25 ng/ml. The intra- and inter-assay coefficients of variation were on average 3.2 and 14.6%, respectively. DNA content was measured by the spectrophotometric method (Labarca and Paigen, 1980), and used to standardize the results.

Statistical analysis

All experimental data are shown as the mean ±SEM of values obtained in separate experiments. The statistical significance of differences in the effects of EGF on PGF2α and PGE2 production and the expression of EGF production and *EGFR* mRNA expression in endometrial tissue was assessed by analysis of variance (ANOVA) followed by Tukey-Kramer test for multiple comparisons. Statistical significance of differences in the EGF production and *EGFR* mRNA expression between endometrial epithelial and stromal cells was assessed by Student's *t*-test using GraphPad Prism (GraphPad Software, La Jolla, CA, USA).

Results

EGF concentration in bovine endometrium was significantly higher in the mid luteal stages than in the other luteal stages ($P < 0.05$; Fig. 1A). *EGFR* mRNA expression was greater ($P<0.05$) in the mid and late luteal stages than in the other luteal stages (Fig. 1B).

EGF concentration was higher in epithelial cells than in stromal cells ($P < 0.05$, Fig. 2A). *EGFR* mRNA expression was greater ($P < 0.05$) in the epithelial cells than in the stromal cells (Fig. 2B).

EGF in doses 10 and 100 nm increased ($P < 0.05$) PGF2α production (Fig. 3A), but 100 nm dose increased ($P < 0.05$) PGE2 production (Fig. 3B) by endometrial

epithelial cells compared to untreated controls (0 dose). On the other hand, 100 nm dose of EGF increased ($P < 0.05$) PGF2α (Fig. 3C) but not PGE2 (Fig. 3D), production by endometrial stromal cells.

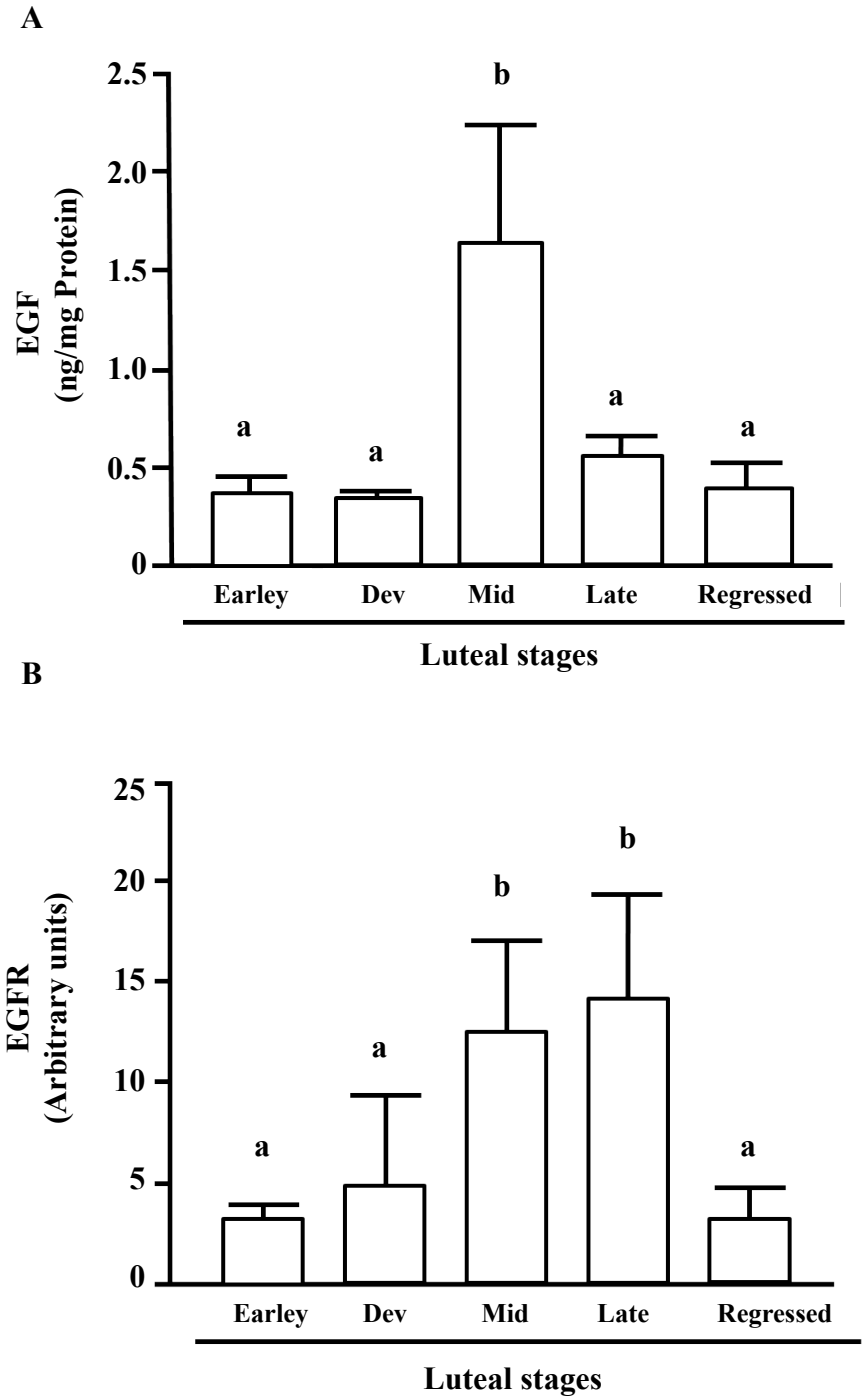

Figure 1. Concentrations of epidermal growth factor (EGF) protein (mean ± SEM, n = 3 cows/stage; Fig. 1A) and expression of EGF receptor (EGFR) mRNA (mean ± SEM, n = 5 cows/stage; Fig. 1B) in bovine endometrial tissue during the luteal stages. Endometrial tissues were collected from cows in five different luteal stages. Different superscript letters indicate significant differences ($P < 0.05$) in the concentration of EGF and EGFR mRNA at different luteal stages, as determined by ANOVA followed by Tukey's multiple comparison test.

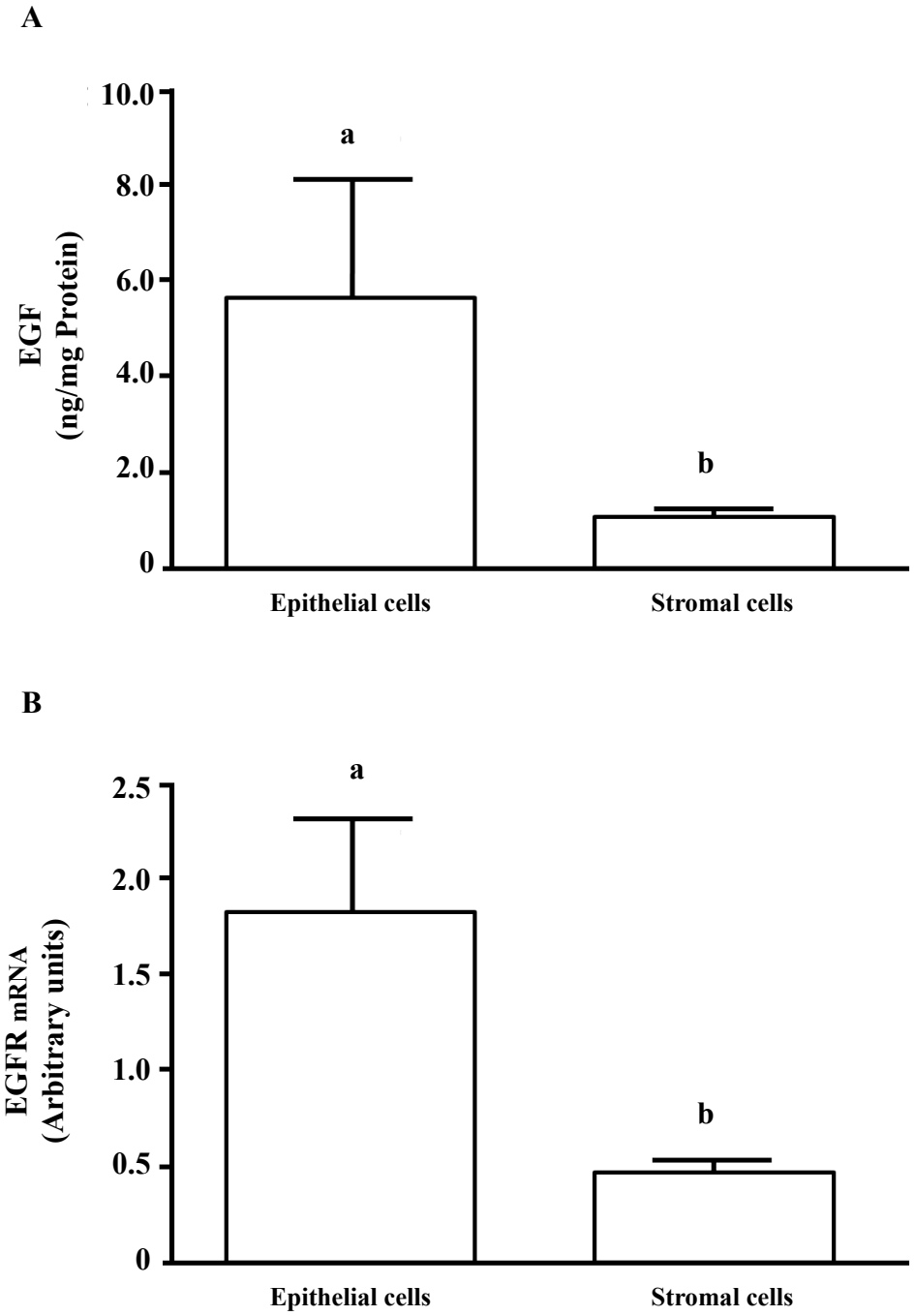

Figure 2. Concentration of epidermal growth factor (EGF) protein (mean ± SEM; Fig. 2A) in the cultured bovine endometrial epithelial (n = 3 cows) and stromal (n = 3 cows) cells. Expression of EGF receptor (EGFR) mRNA (mean ± SEM; Fig. 2B) in the cultured bovine endometrial epithelial (n = 4 cows) and stromal (n = 4 cows) cells. Cells used in the experiment in Fig. 2 were isolated between day 0 and day 4 post-ovulation from 14 cows. Different superscript letters indicate significant differences ($P < 0.05$) between epithelial cells and stromal cells, as determined by unpaired *t* test.

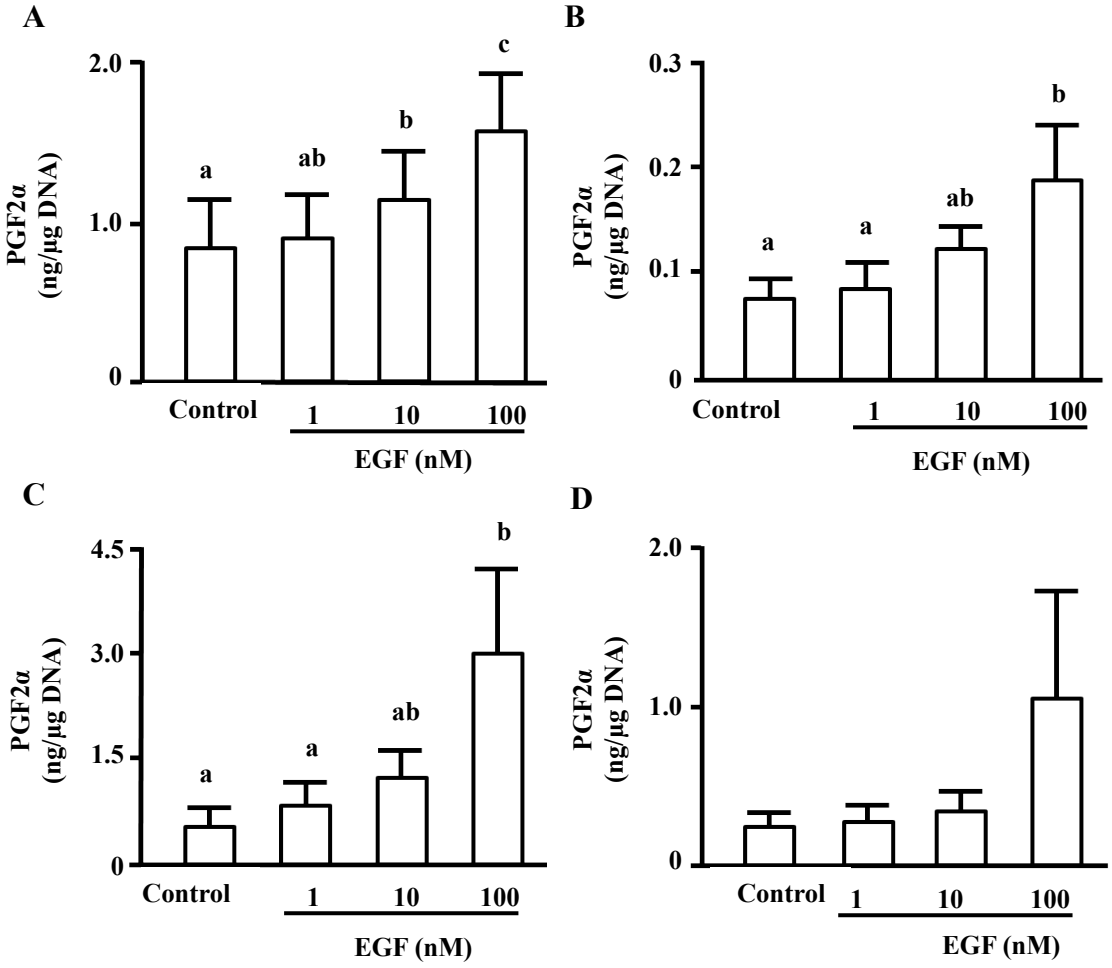

Figure 3. Effects of epidermal growth factor (EGF) on prostaglandin F2α (PGF2α) and prostaglandin E2 (PGE2) synthesis by cultured bovine endometrial epithelial (n = 4 cows; Fig. 3A, 3B) and stromal (n = 4 cows) cells (Fig. 3C, 3D). Cells used in the experiment in Fig. 3 were isolated between day 0 and day 4 post-ovulation from 8 cows. The cells were treated with EGF (1, 10, 100 nm) for 24 h (mean ± SEM, n = 4 experiments. Each experiment was performed in triplicate). Different superscript letters indicate significant differences ($P < 0.05$), as determined by ANOVA followed by Tukey's multiple comparison tests.

Discussion

The results of the present study demonstrate that EGF is mainly produced by uterine epithelial cells, and that it has the ability to increase PGF2α and PGE2 production in both epithelial and stromal cells, and therefore may locally play a role in regulating uterine function throughout luteal stages.

Epidermal growth factor protein and *EGFR* mRNA were expressed in the bovine endometrium throughout the estrous cycle. The protein concentration of EGF was significantly higher in the mid luteal stage (Fig. 1A). It is well known that the biological action of EGF is mediated by EGFR (Wong and Guillaud, 2004). *EGFR* mRNA expression was higher in the mid and late luteal stages (Fig. 1B). Ovarian steroids including progesterone (P4) were found to up-regulate the expressions of EGF family genes in mice (Das *et al*., 1995; Reese *et al*., 1998). Since P4 concentration is highest in the mid luteal stages, P4 may also have a stimulatory effect on EGF and EGFR in bovine endometrium. The results of the present study that EGF protein concentration was higher in the mid luteal stage (days 8-12) in endometrium tissue differs from those of Katagiri and Takahashi (2004), in which EGF concentration was higher on days 2-4 and days 13-14 than on days 5-11 in bovine endometrial tissue (Katagiri and Takahashi, 2004). The same authors reported similar changes in both sides of uterine horns between days 2 and 20 of the estrous cycle in cows (Katagiri and Takahashi, 2004), however, a previous study reported different progesterone and PGE2 concentrations between uterine horns ipsilateral and contralateral to the corpus luteum in cattle (Cerbito *et al*., 1994), thus, endometrial tissue from the ipsilateral horn to the corpus luteum were used in the present study. Previous studies

demonstrated that EGF stimulates PGE2 production in glandular cells and PGE2 and PGF2α production in stromal cells (Zhang *et al*., 1992). In the present study, we observed the stimulation of both PGE2 and PGF2α by EGF. A previous study using cultured porcine endometrial cells showed that PGF2α and PGE2 secretion depends on the type of cell and time of incubation (Blitek and Ziecik, 2004). So, in bovine, PGs secretion may also change depending on the cell type and time of incubation.

Epidermal growth factor regulates several uterine functions including endometrial cell proliferation, cell differentiation and PGs production in several species. These effects are mediated by EGFR present in target cells (Zhang *et al*., 1992; Krishnaswamy *et al*., 2010). The finding that EGF protein concentration was higher in epithelial cells than in stromal cells (Fig. 2A) indicates that EGF is mainly produced by endometrial epithelial cell. Interestingly, endometrial epithelial cells expressed greater *EGFR* mRNA than stromal cells (Fig. 2B). A recent study on the cyclic changes in bovine endometrium showed that the remodeling process is more active in endometrial epithelial cells than in stromal cells (Arai *et al*., 2013). In addition, EGFR was shown to mediate the stimulatory effect of oxytocin on lutueolytic PGF2α production in bovine endometrial epithelial cells (Krishnaswamy *et al*., 2010). In the present study, treating bovine endometrial epithelial cells with EGF increased PGF2α and PGE2 production. Since PGE2 stimulate the expression of pregnancy-related genes prior to pregnancy recognition in ruminants (Spencer *et al*., 2013), the above experimental evidences suggest that EGF regulates not only uterine function during the estrous cycle but also early pregnancy by controlling PGs production.

Repeat breeder cow is defined as the cow that failed to conceive after several inseminations without detectable abnormalities in their genital tracts and with apparently normal estrous cycles (Casida, 1961). The levels of endometrial EGF protein in repeat breeder cows is lower than normal (fertile) cows on days 3 and 14 of the estrous cycle (Katagiri and Takahashi, 2004). Endometritis has also been associated with infertility in cow. Infection with gram-positive bacteria such as *T. Pyogenes* causes loss of endometrial epithelial cells and tissue damage (Amos *et al*., 2014). Our results revealed that EGF and EGFR are higher in epithelial cells than in stromal cells. The above findings suggest that the low concentration of EGF observed in repeat breeder cows is due to a partial loss of epithelial cells during a subclinical inflammatory process. Katagiri and Takahashi (2008) demonstrated that the beneficial effects of estradiol benzoate (5 mg) in a progestin-based treatment are effective for restoration of a normal EGF profile and fertility in repeat breeder cows having lesser endometrial EGF concentrations.

The overall results indicate that 1) the highest amount of EGF is produced by bovine endometrium in the mid-luteal stage, 2) endometrial *EGFR* mRNA expressions are higher in the mid and late-luteal stages than in other stages, 3) EGF is expressed mainly by uterine epithelial cell, and 4) EGF has the ability to increase PGE2 and PGF2α production in both epithelial and stromal cells and therefore may play a role in local regulation of uterine function.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research was supported by a Grant-in-Aid for Scientific Research (No. 26450379) from the Japan Society for the Promotion of Science (JSPS).

References

Akbalik ME, Ketani MA. 2013. Expression of epidermal growth factor receptors and epidermal growth factor, amphiregulin and neuregulin in bovine uteroplacental tissues during gestation. *Placenta*, 34:1232-1242.

Amos MR, Healey GD, Goldstone RJ, Mahan SM, Düvel A, Schuberth HJ, Sandra O, Zieger P, Dieuzy-Labaye I, Smith DG, Sheldon IM. 2014. Differential endometrial cell sensitivity to a cholesterol-dependent cytolysin links Trueperella pyogenes to uterine disease in cattle. *Biol Reprod*, 90:1-13.

Arai M, Yoshioka S, Tasaki Y, Okuda K. 2013. Remodeling of bovine endometrium throughout the estrous cycle. *Anim Reprod Sci*, 142:1-9.

Arosh JA, Parent J, Chapdelaine P, Sirois J, Fortier MA. 2002. Expression of cyclooxygenases 1 and 2 and prostaglandin E synthase in bovine endometrial tissue during the estrous cycle. *Biol Reprod*, 67:161-169.

Blitek A, Ziecik AJ. 2004. Prostaglandins F and E secretion by porcine epithelial and stromal endometrial cells on different days of the oestrous cycle. *Reprod Domest Anim*, 39:340-346.

Casida LE. 1961. Present status of the repeat-breeder cow problem. *J Dairy Sci*, 44:2323-2329.

Cerbito WA, Miyamoto A, Balagapo CR Jr, Natural NG, Miyazawa K, Sato K. 1994. Prostaglandin E2 levels in uterine tissues and its relationship with uterine and luteal progesterone during the estrous cycle in dairy cows. *Theriogenology*, 42:941-950.

Das SK, Chakraborty I, Paria BC, Wang XN, Plowman G, Dey SK. 1995. Amphiregulin is an implantation-specific and progesterone-regulated gene in the mouse uterus. *Mol Endocrinol*, 9:691-705.

Dorniak P, Bazer FW, Spencer TE. 2011. Prostaglandins regulate conceptus elongation and

mediate effects of interferon tau on the ovine uterine endometrium. *Biol Reprod*, 84:1119-1127.

Fortier MA, Guilbault LA, Grasso F. 1988. Specific properties of epithelial and stromal cells from the endometrium of cows. *J Reprod Fertil*, 83:239-248.

Gharib-Hamrouche N, Chêne N, Martal J. 1995. Comparative expression of TGF-alpha and EGF genes in the ovine conceptus and uterine endometrium in the peri-implantation period. *Reprod Nutr Dev*, 35:291-303.

Holmes PV, Gordashko BJ. 1980. Evidence of prostaglandin involvement in blastocyst implantation. *J Embryol Exp Morphol*, 55:109-122.

Katagiri S, Takahashi Y. 2004. Changes in EGF concentrations during estrous cycle in bovine endometrium and their alterations in repeat breeder cows. *Theriogenology*, 62:103-112.

Katagiri S, Takahashi Y. 2006. Potential relationship between normalization of endometrial epidermal growth factor profile and restoration of fertility in repeat breeder cows. *Anim Reprod Sci*, 95:54-66.

Katagiri S, Takahashi Y. 2008. A progestin-based treatment with a high dose of estradiol benzoate normalizes cyclic changes in endometrial EGF concentrations and restores fertility in repeat breeder cows. *J Reprod Dev*, 54:473-479.

Kennedy TG, Brown KD, Vaughan TJ. 1994. Expression of the genes for the epidermal growth factor receptor and its ligands in porcine oviduct and endometrium. *Biol Reprod*, 50:751-756.

Krishnaswamy N, Lacroix-Pepin N, Chapdelaine P, Taniguchi H, Kauffenstein G, Chakravarti A, Danyod G, Fortier MA. 2010. Epidermal growth factor receptor is an obligatory intermediate for oxytocin-induced cyclooxygenase 2 expression and prostaglandin F2alpha production in bovine endometrial epithelial cells. *Endocrinology*, 151:1367-1374.

Labarca C, Paigen K. 1980. A simple, rapid, and sensitive DNA assay procedure. *Anal Biochem*, 102:344-352.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*, 25:402-408.

Malayer JR, Woods VM. 1998. Expression of estrogen receptor and maintenance of hormone-responsive phenotype in bovine fetal uterine cells. *Domest Anim Endocrinol*, 15:141-154.

Miyamoto Y, Skarzynski DJ, Okuda K. 2000. Is tumor necrosis factor alpha a trigger for the initiation of endometrial prostaglandin F(2alpha) release at luteolysis in cattle? *Biol Reprod*, 62:1109-1115.

Murakami S, Shibaya M, Takeuchi K, Skarzynski DJ, Okuda K. 2003. A passage and storage system for isolated bovine endometrial epithelial and stromal cells. *J Reprod Dev*, 49: 531-538.

Oettel M, Koch M, Kurischko A, Schubert K. 1979. Direct evidence for the involvement of prostaglandin F2alpha in the first step of estrone-induced blastocyst implantation in the spayed rat. *Steroids*, 33:1-8.

Okuda K, Miyamoto Y, Skarzynski DJ. 2002. Regulation of endometrial prostaglandin F2alpha synthesis during luteolysis and early pregnancy in cattle. *Domest Anim Endocrinol*, 23:255-264.

Osnes T, Sandstad O, Skar V, Osnes M, Kierulf P. 1993. Total protein in common duct bile measured by acetonitrile precipitation and a micro bicinchoninic acid (BCA) method. *Scand J Clin Lab Invest*, 53:757-763.

Reese J, Brown N, Das SK, Dey SK. 1998. Expression of neu differentiation factor during the periimplantation period in the mouse uterus. *Biol Reprod*, 58:719-727.

Sağsöz H, Ketani MA, Saruhan BG. 2012. Expression of the erbB/HER receptor family in the bovine uterus during the sexual cycle and the relation of this family to serum sex steroids. *Biotech Histochem*, 87:105-116.

Skarzynski DJ, Miyamoto Y, Okuda K. 2000. Production of prostaglandin F2alpha by cultured bovine endometrial cells in response to tumor necrosis factor alpha: cell type specificity and intracellular mechanisms. *Biol Reprod*, 62:1116-1120.

Spencer TE, Forde N, Dorniak P, Hansen TR, Romero JJ, Lonergan P. 2013. Conceptus-derived prostaglandins regulate gene expression in the endometrium prior to pregnancy recognition in ruminants. *Reproduction*, 146:377-387.

Tamada H, Yoh C, Inaba T, Takano H, Kawate N, Sawada T. 2000. Epidermal growth factor (EGF) in the goat uterus: immunohistochemical localization of EGF and EGF receptor and effect of EGF on uterine activity in vivo. *Theriogenology*, 54:159-169.

Tanikawa M, Acosta TJ, Fukui T, Murakami S, Korzekwa A, Skarzynski DJ, Piotrowska KK, Park CK, Okuda K. 2005. Regulation of prostaglandin synthesis by interleukin-1alpha in bovine endometrium during the estrous cycle. *Prostaglandins Other Lipid Mediat*, 78:279-290.

Tanikawa M, Lee HY, Watanabe K, Majewska M, Skarzynski DJ, Park SB, Lee DS, Park CK, Acosta TJ, Okuda K. 2008. Regulation of prostaglandin biosynthesis by interleukin-1 in cultured bovine endometrial cells. *J Endocrinol*, 199:425-434.

Uenoyama Y, Hattori S, Miyake M, Okuda K. 1997. Up-regulation of oxytocin receptors in porcine endometrium by adenosine 3',5'-monophosphate. *Biol Reprod*, 57:723-728.

Weems CW, Weems YS, Randel RD. 2006. Prostaglandins and reproduction in female farm animals. *Vet J*, 171:206-228.

Wong RW, Guillaud L. 2004. The role of epidermal growth factor and its receptors in mammalian CNS. *Cytokine Growth Factor*, 15:147-156.

Zhang Z, Krause M, Davis DL. 1992. Epidermal growth factor receptors in porcine endometrium: binding characteristics and the regulation of prostaglandin E and F2alpha production. *Biol Reprod*, 46:932-936.