REPRODUCTION
RESEARCH

Summer heat stress affects prostaglandin synthesis in the bovine oviduct

Yoshihiko Kobayashi, Kaori Wakamiya, Misa Kohka, Yuki Yamamoto and Kiyoshi Okuda

Laboratory of Reproductive Physiology, Graduate School of Environmental and Life Science, Okayama University, 1-1-1 Tsushimanaka, Okayama, Okayama 700-8530, Japan

Correspondence should be addressed to K Okuda; Email: kokuda@cc.okayama-u.ac.jp

Abstract

Summer heat stress (HS) negatively affects reproductive functions, including prostaglandin (PG) $F_{2\alpha}$ secretion in the endometrium, and decreases fertility in cattle. In the present study, we examined the effects of elevated temperatures on PG synthesis in oviductal epithelial cells. The epithelial cells obtained from the ampulla and isthmus of the oviduct were incubated at various temperatures (38.5, 39.5, 40.0, and 40.5 °C) for 24 h. In the ampulla, PGE_2 concentration was higher at 40.5 °C than at 38.5 °C, while $PGF_{2\alpha}$ production was not affected by the temperatures in this range. The expressions of microsomal PGE synthase 1 (*PTGES* (*mPGES1*)), cytosolic PGES (*PTGES3* (*cPGES*)), and heat shock protein 90 (*HSP90AA1* (*HSP90*)) mRNAs and proteins were higher at 40.5 °C than at 38.5 °C in the ampullary epithelial cells. Seasonal changes in the expressions of *PGES* and *HSP90AA1* mRNAs in oviductal tissues were also investigated. The expressions of *PTGES3* and *HSP90AA1* mRNAs were higher in the ampullary tissues in summer than in winter. In summary, elevated temperatures stimulated PGE_2 production in the ampullary oviduct by increasing the expressions of PGESs and *HSP90AA1*, which can activate cPGES. The overall results suggest that HS upsets PG secretions and reduces oviductal smooth muscle motility, which in turn could decrease gamete/embryo transport through the oviduct.

Reproduction (2013) **146** 103–110

Introduction

In mammals, the oviduct plays roles in the transport of gametes and fertilized oocytes and the development of embryos (Menezo & Guerin 1997, Suarez 2008, Ulbrich *et al*. 2010). After fertilization has occurred in the ampulla of the oviduct, the embryo is transported to the uterus within a few days. The transport to the uterus is caused by waves of contraction and relaxation of the smooth muscle and the ciliation of the oviductal epithelial cells (Halbert *et al*. 1976, Hunter 2012). Prostaglandin (PG) E_2 and $PGF_{2\alpha}$ concentrations in the oviduct ipsilateral to the corpus luteum (CL) or the dominant follicle are highest in the periovulatory phase (Wijayagunawardane *et al*. 1998). These PGs seem to control gamete/embryo transport by stimulating smooth muscle motility (Wijayagunawardane *et al*. 2001).

PG biosynthesis starts from the liberation of arachidonic acid from phospholipids (Okuda *et al*. 2002). Cyclooxygenases convert arachidonic acid to PGH_2, which is the precursor for various PGs including PGE_2 and $PGF_{2\alpha}$ (Garavito *et al*. 2002, Gabler *et al*. 2008). The conversion of PGH_2 to PGE_2 or $PGF_{2\alpha}$ is catalyzed by the specific downstream enzymes PGE synthase (PGES) and PGF synthase (PGFS) respectively (Arosh *et al*. 2002). The bovine oviduct expresses three PGES isozymes: microsomal PGES1 (mPGES1), mPGES2, and cytosolic PGES (cPGES) (Gauvreau *et al*. 2010).

Summer heat stress (HS) weakens estrous behavior, suppresses follicular development, and causes early embryo mortality, all of which decrease the fertility rate in cows (Gwazdauskas *et al*. 1975, Cavestany *et al*. 1985, De Rensis & Scaramuzzi 2003, Sakatani *et al*. 2004, Hansen 2009). HS also increases $PGF_{2\alpha}$ production in the endometrium, which might result in the early regression of CL or the death of embryos (Putney *et al*. 1988, Malayer *et al*. 1990). HS is of special concern to the livestock industry because of recent global warming.

Heat shock proteins (HSPs) are highly expressed under hot conditions and protect cells from various stresses including heat and reactive oxidants (Kregel 2002). In particular, HSP90AA1 (HSP90) is known to activate cPGES, which increases the production of PGE_2 in rat fibroblasts (Tanioka *et al*. 2003). Thus, HS may stimulate PGE_2 production in the oviductal epithelium, which would reduce gamete/embryo transport.

We hypothesized that HS negatively affects PG synthesis in the bovine oviduct. In the present study, to prove the above hypothesis, we investigated the effects of elevated temperatures on the synthesis of PGE_2

ISSN 1470–1626 (paper) 1741–7899 (online)
DOI: 10.1530/REP-12-0479
Online version via www.reproduction-online.org

and $PGF_{2\alpha}$ in cultured bovine oviductal epithelial cells. We also studied the expressions of PGESs and HSP90AA1 in bovine oviducts obtained in winter and summer.

Materials and Methods

Collection of bovine oviducts

Oviducts from Holstein cows were collected at a local abattoir within 10–20 min of exsanguination. The stages of the estrous cycle were determined based on a macroscopic observation of the ovaries and uteri (Okuda *et al*. 1988, Miyamoto *et al*. 2000). After trimming of the oviducts that were ipsilateral to the CL, the ampullary and isthmic sections were immediately frozen and stored at −80 °C until mRNA extraction. For cell culture experiments, the oviducts were submerged in ice-cold saline and transported to the laboratory.

Isolation of oviductal cells

Oviductal tissues collected at days 0–3 after ovulation were utilized for cell culture. The oviduct was separated into infundibulum, ampulla, ampullary–isthmic junction, isthmus, and utero–tubal junction sections (Fig. 1). The epithelial cells were isolated from the ampullary and isthmic sections of the oviduct. A Teflon catheter (internal diameter: 0.5 mm) was inserted into the ampullary and isthmic sections of the oviduct, and the luminal wall was washed five times with 1 ml of sterile Hank's balanced salt solution (HBSS) containing 0.1% (wt/vol) BSA (Roche), 100 IU/ml penicillin (Meiji Seika Pharma, Tokyo, Japan), and 100 μg/ml streptomycin (Meiji Seika Pharma). The oviduct was connected to a peristaltic pump (Gilson, Middleton, WI, USA) and perfused with 20 ml of sterile HBSS containing 0.25% (wt/vol) bovine trypsin (>7500 BAEE units/mg solid; Sigma–Aldrich), 0.02% (wt/vol) EDTA2Na (Sigma–Aldrich), 0.1% (wt/vol) BSA, 100 IU/ml penicillin, and 100 μg/ml streptomycin (at a flow rate of 10 ml/min, 38 °C, for 30 min). After perfusion, the dissociated epithelial cells were filtered through metal meshes (150 and 77 μm) to remove undissociated tissue fragments. The filtrates were washed by centrifugation (180 ***g*** for 10 min at 4 °C) with Tris-buffered ammonium chloride (pH 7.5) to remove hemocytes and with DMEM (Sigma–Aldrich) supplemented with 0.1% (wt/vol) BSA, 100 IU/ml penicillin, and 100 μg/ml streptomycin. After the washes, the cells were counted using a hemocytometer. Cell viability was >95% as assessed by 0.5% (wt/vol) trypan blue dye exclusion. The final pellets of the epithelial cells were resuspended in DF (DMEM/Ham's F-12; 1:1 (vol/vol) (Invitrogen) supplemented with 10% (vol/vol) bovine serum (Invitrogen), 20 mg/ml gentamicin (Invitrogen), and 2 mg/ml amphotericin B (Sigma–Aldrich)). These cells were seeded at a density of 1.0×10^5 viable cells/ml in 25 cm^2 culture flasks (Greiner Bio-One, Frickenhausen, Germany) and cultured at 38.5 °C in a humidified atmosphere of 5% CO_2 in air. Because the epithelial cells attached 24–48 h after plating, the medium in the epithelial cell culture was replaced 48 h after plating. The medium was changed every 2 days until confluence was reached.

To purify the epithelial cells, the cells were trypsinized on reaching sub-confluence. Briefly, the cells in the culture flasks were washed with PBS (−) twice. After washing, 0.02% (wt/vol) porcine trypsin (1000–2000 BAEE units/mg solid; Sigma–Aldrich) with 0.008% (wt/vol) EDTA (Sigma–Aldrich) in PBS was added to the flasks, and the cells were incubated for 5 min at 38.5 °C to detach stromal cells. Then, the solution containing the stromal cells was removed, and the remaining cells were incubated with 2 mmol/l EDTA in PBS for 2 min at 38.5 °C. After incubation, the solution was removed and the cells were washed with PBS (−), and the cells were incubated for 10 min at 38.5 °C with 0.02% (wt/vol) bovine trypsin in PBS. After incubation, the cells were washed by centrifugation (180 ***g*** for 10 min at 4 °C) with the culture medium. Then, the cells were placed in fresh DF to adjust them to a density of 1.0×10^5 viable cells/ml. These cells were seeded in 4-well plates (Thermo Fisher Scientific, Waltham, MA, USA), 48-well plates (Greiner Bio-One), and 75 cm^2 culture flasks (Greiner Bio-One) and cultured at 38.5 °C in a humidified atmosphere of 5% CO_2 in air. The medium was changed every 48 h until confluence was reached. When the cells reached confluence (10–11 days after starting the culture), they were used for experiments.

The homogeneity of the epithelial cells was evaluated using immunofluorescence staining for specific markers of epithelial (cytokeratin) and stromal (vimentin) cells as described previously (Malayer & Woods 1998, Tanikawa *et al*. 2008) with our modification. Briefly, oviductal epithelial and endometrial stromal (control) cells were seeded on collagen-coated sterile cover glasses in six-well plates at a density of 5.0×10^4 cells/ml. The endometrial stromal cells were isolated as described previously (Tanikawa *et al*. 2008). The cells were fixed with 4% (wt/vol) paraformaldehyde (Nacalai Tesque, Inc., Kyoto, Japan) for 10 min 48 h after seeding. Then, the cells were incubated with 5% (wt/vol) skim milk (Nacalai Tesque, Inc.) in PBS containing 0.1% (vol/vol) Tween-20 (PBS-T) for 60 min at room temperature. After blocking, the cells were

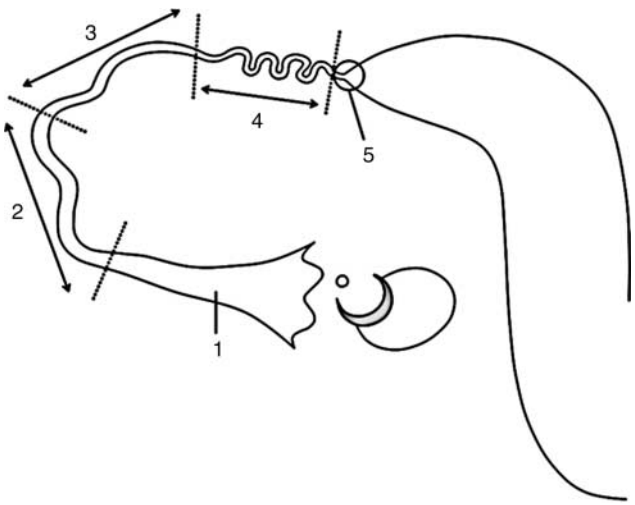

Figure 1 Five sections into which the oviducts were separated (1, infundibulum; 2, ampulla; 3, ampullary–isthmic junction; 4, isthmus; and 5, utero–tubal junction). The ampulla and isthmus sections were utilized for mRNA determination and epithelial cell culture.

incubated with specific primary antibodies to cytokeratin (anti-cytokeratin-IgG-mouse; Sigma–Aldrich, 1:500 dilution) or vimentin (anti-vimentin-IgG-mouse; Sigma–Aldrich, 1:5000 dilution) in 5% (wt/vol) skim milk overnight at 4 °C. After incubation, the cells were incubated again with secondary antibodies (anti-mouse-IgG Alexa 594 conjugate-donkey; Sigma–Aldrich; 1:500 dilution for cytokeratin 18; anti-mouse-IgG FITC conjugate-donkey; Sigma–Aldrich; 1:500 dilution for vimentin, in PBS-T) for 60 min at room temperature and mounted with ProLong Gold Antifade Reagent with DAPI (Invitrogen) on slide glasses. Fluorescence was observed using a fluorescence microscope (Olympus, Tokyo, Japan). In the epithelial cell culture, contamination of the oviductal stromal cells was <1% (Fig. 2).

Experiment 1: effects of various temperatures on PG production in cultured bovine oviductal epithelial cells

Epithelial cells that reached confluence in the four-well plates were used for this experiment. The cells were incubated with serum-free DF (DMEM/Ham's F-12 1:1 (vol/vol) supplemented with 0.1% BSA, 5 μg/ml holo-Transferrin (Sigma–Aldrich), and 2 μg/ml insulin (Sigma–Aldrich)) at 38.5, 39.5, 40.0, and 40.5 °C for 24 h. After incubation, the medium was collected in a 1.5 ml tube containing a 1% stabilizer solution (0.3 mol/l EDTA and 1% (w/v) acetylsalicylic acid, pH 7.3), and the concentrations of PGE_2 and $PGF_{2\alpha}$ in the medium were measured by enzyme immunoassay. The cells were collected for the measurement of DNA amount by DNA assay.

Experiment 2: effects of elevated temperatures on PGES *mRNA expressions in cultured bovine oviductal epithelial cells*

Epithelial cells that reached confluence on the 48-well plates were used for this experiment. The cells were incubated with serum-free DF at 38.5 and 40.5 °C for 24 h. After incubation, the cells were collected and used for the investigation of the expressions of *PTGES*, *PTGES2*, and *PTGES3* mRNAs.

Experiment 3: comparison of mRNA expression of PGESs *and* HSP90AA1 *in summer and winter in bovine oviductal tissues*

For the determination of the expressions of *PGES* and *HSP90AA1* mRNAs, oviductal tissues (days 0–6 after ovulation) were collected from 14 cows in winter (November–March, 2011–2012) and 14 cows in summer (July–September, 2012). The cows were slaughtered at a local abattoir in Okayama, Japan. Average temperatures in Okayama during the sampling periods were 5.3 °C in winter and 27.5 °C in summer. The range of temperatures during the summer sampling period was 22.1–36.8 °C. Although the average temperature is reported as 27.5 °C herein, there were 33 days (of 46 days) when the daily maximum temperature was >33 °C. This climate seems to be under an apparent HS condition for dairy cows. The temperature data were obtained from Japan Meteorological Agency.

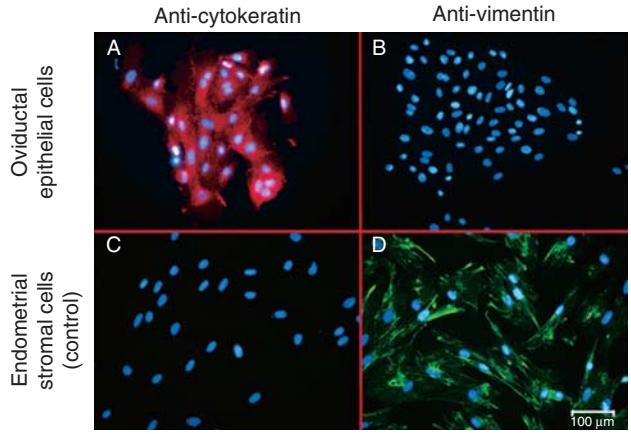

Figure 2 Representative photomicrographs of immunostaining with anti-cytokeratin and anti-vimentin antibodies in ampullary oviductal epithelial cells (A and B) and endometrial stromal cells (C and D). Alexa 594 (red) was used for staining cytokeratin and FITC (green) was used for staining vimentin as the secondary antibody. DAPI (blue) was used to visualize nuclei. Scale is the same in all the photomicrographs. Staining in the isthmus was virtually the same as that in the ampulla.

Experiment 4: effects of elevated temperatures on HSP90AA1 mRNA and protein expressions in cultured bovine oviductal epithelial cells

Epithelial cells that reached confluence on the 48-well plates and in the 75 cm^2 culture flasks were used for this experiment. The cells were incubated with a serum-free medium at 38.5 and 40.5 °C for 24 h. After incubation, the cells were collected and used to investigate *HSP90AA1* mRNA and protein expressions.

Enzyme immunoassay

The concentrations of PGE_2 and $PGF_{2\alpha}$ in the culture medium were determined by an enzyme immunoassay as described previously (Uenoyama *et al.* 1997, Tanikawa *et al.* 2005). The PGE_2 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, 2.6 and 8.4% respectively. The $PGF_{2\alpha}$ 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, 3.9 and 17.7% respectively. DNA content was measured by the spectrophotometric method (Labarca & Paigen 1980) and used to standardize the results.

Total RNA extraction and quantitative RT-PCR

Total RNA was extracted from the oviductal tissues and cells using TRIsure (Bioline, London, UK) according to the manufacturer's directions. Using iScript RT Supermix for RT-qPCR (Bio-Rad Laboratories), 1 μg of each total RNA was reverse-transcribed. Quantifications of mRNA expressions were done with Quantitative RT-PCR using MyiQ (Bio-Rad Laboratories) and SsoAdvanced SYBR Green Supermix (Bio-Rad Laboratories) starting with 2 ng of reverse-transcribed

Table 1 Primer sequences used for quantitative RT-PCR analysis.

Genes	Forward and reverse primers	Accession no.	Products (bp)
HSP90AA1	F: 5′-GTATGGACAATGACTCCAATCAAGT-3′ R: 5′-CCGTTTGTTGTAAGGTGTGTATGTA-3′	NM001012670.2	277
PTGES	F: 5′-AGGACGCTCAGAGACATGGA-3′ R: 5′-TTCGGTCCGAGGAAAGAGTA-3′	NM174443	142
PTGES2	F: 5′-CCTACAGAAAGGTGCCCATC-3′ R: 5′-TGCCCTGACACCAGATAGGT-3′	AY692441	109
PTGES3	F: 5′-AAGGAGAATCTGGCCAGTCA-3′ R: 5′-TCGGAATCATCTTCCCAGTC-3′	AY692440	106
GADPH	F: 5′-CACCCTCAAGATTGTCAGCA-3′ R: 5′-GGTCATAAGTCCCTCCACGA-3′	BC102589	103
ACTB	F: 5′-CAGCAAGCAGGAGTACGATG-3′ R: 5′-AGCCATGCCAATCTCATCTC-3′	AY141970	137
RNA18S1	F: 5′-TCGCGGAAGGATTTAAAGTG-3′ R: 5′-AAACGGCTACCACATCCAAG-3′	AY779625	141

total RNA as described previously (Sakumoto *et al*. 2006). All primers were designed to amplify a product as shown in Table 1, and the specificity of each primer set was confirmed by running the PCR products on a 2.0% agarose gel. Protocol conditions consisted of denaturation at 95 °C for 3 min, followed by 45 cycles at 94 °C for 15 s, 60 °C for 20 s, and 72 °C for 15 s with a final dissociation (melting) curve analysis. To standardize the relative level of the expression of each mRNA, three potential housekeeping genes, β-actin (*ACTB*), 18S rRNA (*RNA18S1*), and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), were initially tested. The expression of *GAPDH* mRNA was most stable among those of the three genes, so the transcripts were selected as internal controls in our experiments. To analyze the relative level of the expression of each mRNA, the $2^{-\Delta\Delta}CT$ method was used (Livak & Schmittgen 2001).

Western blotting

Each protein in the cultured bovine oviductal cells was detected by western blotting analysis as described previously (Nishimura *et al*. 2008). Briefly, the cultured cells were lysed in 200 μl lysis buffer, and the protein concentrations were determined by the BCA method (Osnes *et al*. 1993). The proteins were heated with a SDS gel-loading buffer containing 1% (v/v) β-mercaptoethanol (Wako Pure Chemical Industries Ltd., Osaka, Japan) at 95 °C for 10 min. The samples (50 μg protein) were loaded on 10% (v/v) SDS–PAGE (200 V, 80 min) gel and transblotted onto a 0.2 μm nitrocellulose membrane (GE Healthcare, Milwaukee, WI, USA; 250 mA, 180 min). The membrane was then incubated in PVDF Blocking Reagent for Can Get Signal (Toyobo, Osaka, Japan) for 60 min at room temperature. After blocking, the membrane was cut into two pieces, and each piece was incubated separately with specific primary antibodies to HSP90AA1 (anti-HSP90-IgG-mouse; Abcam, Cambridge, UK, 1:1000 dilution) and ACTB (anti-β-actin-IgG-mouse; Sigma–Aldrich, 1:20 000 dilution) in Can Get Signal Immunoreaction Enhancer Solution 1 (Toyobo) overnight at 4 °C. After incubation, the membrane pieces were incubated again with a secondary antibody (anti-mouse, HRP-linked whole antibody produced in sheep; GE Healthcare, 1:5000 dilution for HSP90AA1 and 1:40 000 dilution for ACTB) in Can Get Signal Immunoreaction Enhancer Solution 2 for 60 min at room temperature. The signal was detected using the ECL Western Blotting Detection System (GE Healthcare), and the intensity of the immunological reaction mixture was estimated by measuring the optical density in the defined area by computerized densitometry using Image J (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

All the experimental data are reported as mean ± S.E.M. The data of PG production are reported as a percentage of control. The statistical significance of differences was assessed by ANOVA followed by Bonferroni/Dunn test for multiple comparisons using StatView (SAS Institute, Cary, NC, USA).

Results

Experiment 1: effects of various temperatures on PG production in cultured bovine oviductal epithelial cells

Temperature affected PGE_2 production in the cultured ampullary epithelial cells. PGE_2 concentration was significantly higher at 40.5 °C than at 38.5 °C (Fig. 3A).

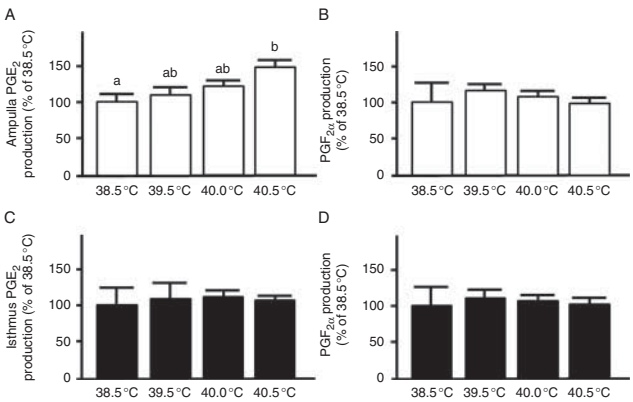

Figure 3 Effects of temperatures (38.5, 39.5, 40.0, and 40.5 °C) on the productions of prostaglandin (PG) E_2 (A and C) and $PGF_{2\alpha}$ (B and D) in epithelial cells collected from the ampulla (white bars) and isthmus (black bars) of the bovine oviduct (mean ± S.E.M., $n=4$ oviducts). Different superscript letters indicate a significant difference ($P<0.05$; ANOVA).

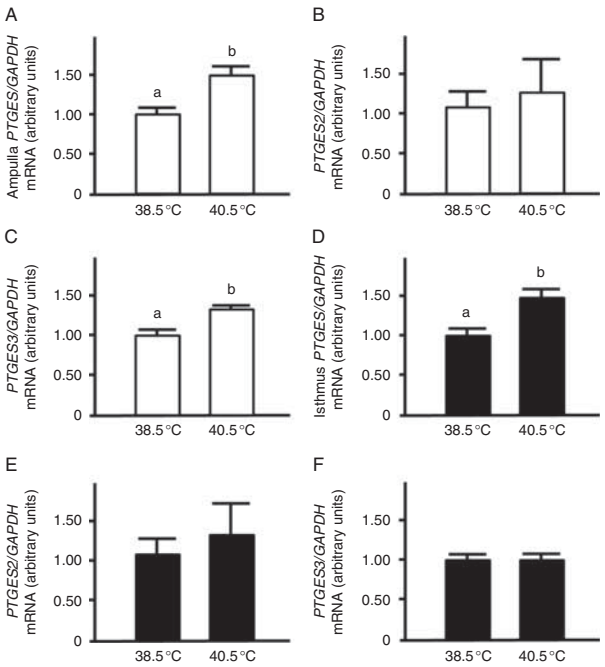

Figure 4 Effects of an elevated temperature (40.5 °C) on the expression of microsomal PGE synthase 1 (*PTGES* (*mPGES1*)) (A and D), *PTGES2* (B and E), and cytosolic PGES (*PTGES3* (*cPGES*)) (C and F) mRNAs in epithelial cells collected from the ampulla (white bars) and isthmus (black bars) of the bovine oviduct (mean ± S.E.M., $n=4$ oviducts). Different superscript letters indicate a significant difference ($P<0.05$; ANOVA).

On the other hand, elevated temperatures did not affect PGE_2 production in the isthmic epithelial cells (Fig. 3B). The culture temperature had no effect on $PGF_{2\alpha}$ production in either the ampullary cells or isthmic cells (Fig. 3C and D). In the experiments described below, the cells were incubated at 38.5 and 40.5 °C, because PGE_2 production at these temperatures is significantly different.

Experiment 2: effects of elevated temperatures on PGES *mRNA expressions in cultured bovine oviductal epithelial cells*

The expressions of *PTGES* (*mPGES1*) and *PTGES3* (*cPGES*) mRNAs were higher at 40.5 °C than at 38.5 °C in the cultured ampullary epithelial cells (Fig. 4A and C; $P<0.05$). The expression of *PTGES2* mRNA was not affected by elevated temperatures (Fig. 4B; $P>0.05$). The expression of *PTGES* mRNA was higher at 40.5 °C than at 38.5 °C in the isthmus (Fig. 4D; $P<0.05$). On the other hand, *PTGES2* and *PTGES3* transcripts were not affected by elevated temperatures (Fig. 4E and F; $P>0.05$).

Experiment 3: comparison of mRNA expressions of PGESs *and* HSP90AA1 *in summer and winter in bovine oviductal tissues*

The expression of *PTGES3* mRNA in the bovine ampullary oviduct was higher in summer than in winter (Fig. 5C; $P<0.05$). In contrast, the mRNA expressions of *PTGES* and *PTGES2* in the ampulla and all *PGES*s in the isthmus were not significantly different in summer and winter (Fig. 5; $P>0.05$). The expression of *HSP90AA1* mRNA in the bovine ampullary oviduct was higher in summer than in winter (Fig. 6A; $P<0.05$). On the other hand, the expression of *HSP90AA1* mRNA in the isthmic cells was not significantly different in summer and winter (Fig. 6B; $P>0.05$).

Experiment 4: effect of elevated temperatures on HSP90AA1 mRNA and protein expressions in cultured bovine oviductal epithelial cells

The expression of *HSP90AA1* mRNA was higher at 40.5 °C than at 38.5 °C in the cultured ampullary (Fig. 7A; $P<0.01$) and isthmic (Fig. 7B; $P<0.05$) oviductal epithelial cells. The expression of HSP90AA1 protein was also higher at 40.5 °C than at 38.5 °C in the cultured cells obtained from both sections of the oviductal epithelium (Fig. 7C and D; $P<0.05$).

Discussion

In the present study, elevated temperatures promoted PGE_2 production, but did not affect $PGF_{2\alpha}$ production in the cultured ampullary oviductal epithelial cells (Fig. 3). The body temperature of cows is ∼38.5 °C, but it

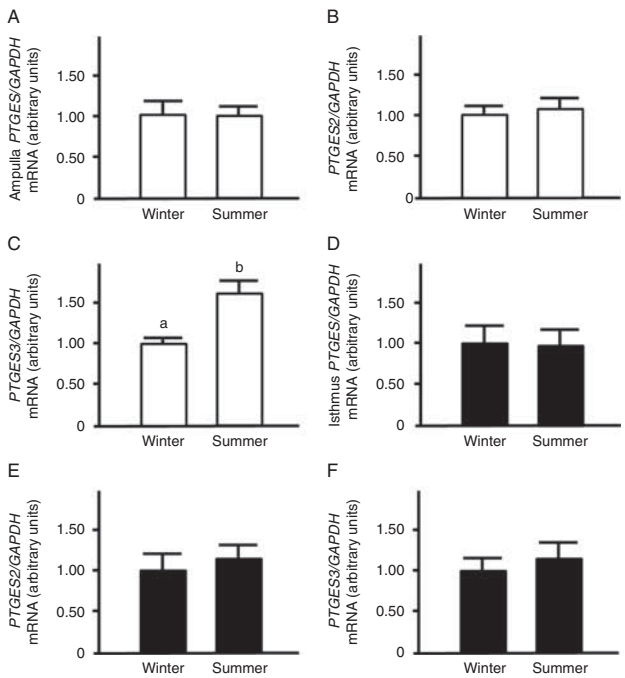

Figure 5 Expressions of *PTGES* (A and D), *PTGES2* (B and E), and *PTGES3* (C and F) mRNAs in the bovine ampullary (white bars) and isthmic (black bars) oviducts (mean ± S.E.M.) in the postovulatory phase in winter (November 21 2011–March 1 2012; $n=14$) and summer (July 23–September 6, 2012, $n=14$). Different superscript letters indicate a significant difference ($P<0.05$; ANOVA).

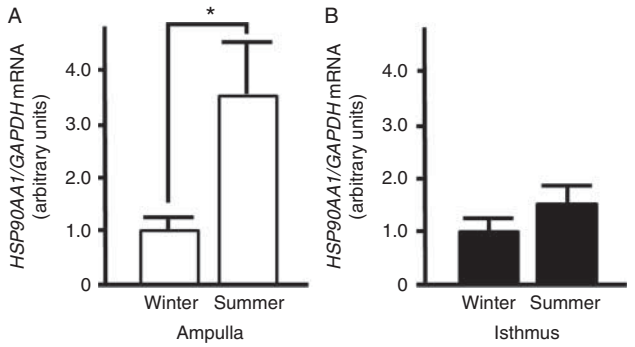

Figure 6 Expression of *HSP90AA1* mRNA in the bovine ampullary (A) and isthmic (B) oviducts (mean±S.E.M.) in the postovulatory phase in winter (November 21 2011–March 1 2012; $n=14$) and summer (July 23–September 6, 2012, $n=14$). The asterisk indicates a significant difference ($P<0.05$; ANOVA).

increases under hot conditions. For example, the vaginal temperature of cows was found to increase to ∼40.5 °C under high-temperature conditions (Nabenishi *et al.* 2011). Oviductal smooth muscle is relaxed by PGE_2 and induced to contract by $PGF_{2\alpha}$ (Al-Alem *et al.* 2007, Siemieniuch *et al.* 2009). Since gametes and embryos are transported by waves of contraction and relaxation, upsetting the balance between PGE_2 and $PGF_{2\alpha}$ secretions by HS may negatively affect the transport.

Elevated temperatures increased PGE_2 production in the ampullary epithelial cells (Fig. 3). The increased PGE_2 production could be due to either increases in the expressions of PGESs or increases in the PGES activity. Mammals have three PGES isozymes: mPGES1, mPGES2, and cPGES (Murakami *et al.* 2002). In the present study, the expressions of *PTGES* and *PTGES3* mRNAs were higher at 40.5 °C than at 38.5 °C in the ampullary epithelial cells (Fig. 4). However, only the expression of *PTGES3* mRNA was higher in summer than in winter in the ampullary oviductal tissue (Fig. 5). Thus, cPGES may play an important role in the stimulation of PGE_2 synthesis in the ampullary oviduct under HS. Since PGE_2 is known to relax the oviductal smooth muscle (Al-Alem *et al.* 2007, Siemieniuch *et al.* 2009), HS may be a cause of summer infertility by decreasing the oviductal motility via the promotion of PGE_2 production. The expression of *PTGES* mRNA in both the oviductal sections did not show any difference in summer and winter (Fig. 5) in contrast to that observed in the *in vitro* experiment (Fig. 4). This discrepancy may be due to the different *in vivo* and *in vitro* conditions. However, since the expression of PTGES3 was increased by HS both *in vivo* and *in vitro*, we believe that summer HS increases PGE_2 production in bovine oviducts.

The expression of HSP90AA1 in the oviduct was higher in summer than in winter (Fig. 6) and was increased by elevated culture temperatures (Fig. 7). HSPs protect cells from various stresses including heat and reactive oxidants (Kregel 2002). HSPs are highly expressed at high temperatures and help the folding of proteins to prevent protein denaturation (Kregel 2002). In particular, HSP90AA1 plays crucial roles as a molecular chaperone in the activation of various client proteins including cPGES (Pearl & Prodromou 2006). In rat fibroblast cells, HSP90AA1 activates cPGES and consequently promotes PGE_2 production (Tanioka *et al.* 2003). Therefore, it is possible that HS increases the expression of HSP90AA1 and the activity of cPGES, resulting in an increase in PGE_2 production in bovine oviductal epithelial cells. Furthermore, the expression of HSP90AA1 in summer could be assumed to increase not only in the oviduct but also in other organs. Since HSP90AA1 is an important chaperone for the actions of various client proteins (Pearl & Prodromou 2006), HS may affect not only oviductal functions but also other body conditions. The expressions of *HSP90AA1* and *PTGES3* mRNAs in the ampullary oviduct were higher in summer than in winter, whereas there was no difference in them in the isthmic oviduct in summer and winter. It has been reported that the expression of HSPs is higher in porcine oocytes in summer than in winter, although there are no seasonal changes in porcine cumulus cell (Pennarossa *et al.* 2012). Therefore, it is possible that the expressions of HSP90AA1 and PTGES3 in the isthmic oviduct are not affected by different seasonal conditions in contrast to those in the ampullary oviduct.

Elevated temperatures did not affect PGE_2 production in the isthmic epithelial cells (Fig. 3), although they stimulated the expression of *PTGES* mRNA (Fig. 4). In periovulatory cows, the expression of PTGES is lower in the isthmus of the oviduct than in the ampulla (Gauvreau

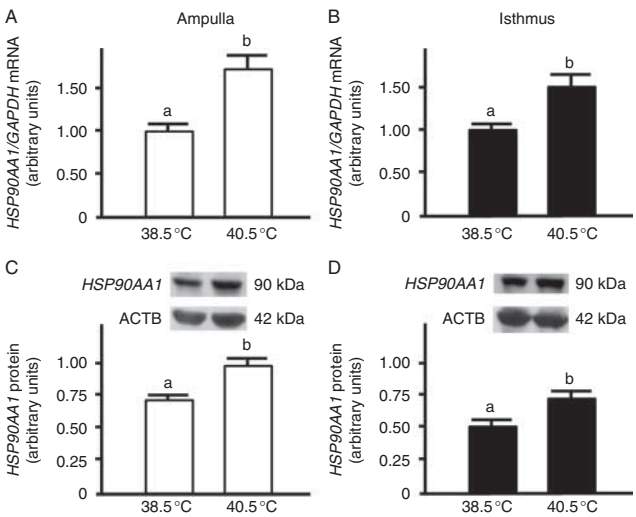

Figure 7 Effects of an elevated temperature (40.5 °C) on the expression of *HSP90AA1* mRNA (A and B, $n=7$ oviducts) and protein (C and D, $n=4$ oviducts) in the epithelial cells collected from the ampulla (white bars) and isthmus (black bars) of the bovine oviduct (mean± S.E.M.). Different superscript letters indicate a significant difference ($P<0.05$; ANOVA).

et al. 2010). Thus, mPGES1 may contribute less to PGES activity in the isthmus than the other two PGESs. Additionally, since the culture temperature did not affect the expression of *PTGES3* mRNA (Fig. 4), PGE_2 production may be at a stable level in the isthmic oviductal epithelium. Mammals have two types of oviductal epithelial cells, ciliated and secretory, and the proportion of ciliated cells to secretary cells is different among the sections of the oviduct (Kölle *et al*. 2009). Thus, PGE_2 production seems to be not affected by elevated temperatures in the isthmus due to the different cell type populations. In conclusion, HS is suggested to not affect the secretary function of PGs in the isthmic oviduct.

The overall findings suggest that HS upsets the balance between PGE_2 and $PGF_{2\alpha}$ secretions, which would be expected to decrease the motility of the oviductal smooth muscle, which is important for gamete/embryo transport. This could lead to the low fertility in cattle in summer.

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 work was supported by Grants-in-Aid for Research Program on Innovative Technologies for Animal Breeding, Reproduction, and Vaccine Development (REP-1002) from the Ministry of Agriculture, Forestry, and Fisheries of Japan and for Scientific Research (No. 24380155) of Japan Society for the Promotion of Science (JSPS).

Acknowledgements

We are grateful to Dr Seiji Ito (Kansai Medical University, Osaka, Japan) for providing the antisera of PGE_2 and $PGF_{2\alpha}$.

References

Al-Alem L, Bridges P, Su W, Gong M, Iglarz M & Ko C 2007 Endothelin-2 induces oviductal contraction via endothelin receptor subtype A in rats. *Journal of Endocrinology* **193** 383–391. (doi:10.1677/JOE-07-0089)

Arosh J, Parent J, Chapdelaine P, Sirois J & Fortier M 2002 Expression of cyclooxygenases 1 and 2 and prostaglandin E synthase in bovine endometrial tissue during the estrous cycle. *Biology of Reproduction* **67** 161–169. (doi:10.1095/biolreprod67.1.161)

Cavestany D, el-Wishy A & Foote R 1985 Effect of season and high environmental temperature on fertility of Holstein cattle. *Journal of Dairy Science* **68** 1471–1478. (doi:10.3168/jds.S0022-0302(85)80985-1)

De Rensis F & Scaramuzzi R 2003 Heat stress and seasonal effects on reproduction in the dairy cow – a review. *Theriogenology* **60** 1139–1151. (doi:10.1016/S0093-691X(03)00126-2)

Gabler C, Odau S, Muller K, Schon J, Bondzio A & Einspanier R 2008 Exploring cumulus–oocyte-complex-oviductal cell interactions: gene profiling in the bovine oviduct. *Journal of Physiology and Pharmacology* **59** (Suppl 9) 29–42.

Garavito R, Malkowski M & DeWitt D 2002 The structures of prostaglandin endoperoxide H synthases-1 and -2. *Prostaglandins and Other Lipid Mediators* **68–69** 129–152. (doi:10.1016/S0090-6980(02)00026-6)

Gauvreau D, Moisan V, Roy M, Fortier M & Bilodeau JF 2010 Expression of prostaglandin E synthases in the bovine oviduct. *Theriogenology* **73** 103–111. (doi:10.1016/j.theriogenology.2009.08.006)

Gwazdauskas F, Wilcox C & Thatcher W 1975 Environmental and managemental factors affecting conception rate in a subtropical climate. *Journal of Dairy Science* **58** 88–92. (doi:10.3168/jds.S0022-0302(75)84523-1)

Halbert S, Tam P & Blandau R 1976 Egg transport in the rabbit oviduct: the roles of cilia and muscle. *Science* **191** 1052–1053. (doi:10.1126/science.1251215)

Hansen P 2009 Effects of heat stress on mammalian reproduction. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **364** 3341–3350. (doi:10.1098/rstb.2009.0131)

Hunter R 2012 Components of oviduct physiology in eutherian mammals. *Biological Reviews of the Cambridge Philosophical Society* **87** 244–255. (doi:10.1111/j.1469-185X.2011.00196.x)

Kölle S, Dubielzig S, Reese S, Wehrend A, König P & Kummer W 2009 Ciliary transport, gamete interaction, and effects of the early embryo in the oviduct: *ex vivo* analyses using a new digital videomicroscopic system in the cow. *Biology of Reproduction* **81** 267–274. (doi:10.1095/biolreprod.108.073874)

Kregel K 2002 Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *Journal of Applied Physiology* **92** 2177–2186. (doi:10.1152/japplphysiol.01267.2001)

Labarca C & Paigen K 1980 A simple, rapid, and sensitive DNA assay procedure. *Analytical Biochemistry* **102** 344–352. (doi:10.1016/0003-2697(80)90165-7)

Livak K & Schmittgen T 2001 Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods* **25** 402–408. (doi:10.1006/meth.2001.1262)

Malayer J & Woods V 1998 Expression of estrogen receptor and maintenance of hormone-responsive phenotype in bovine fetal uterine cells. *Domestic Animal Endocrinology* **15** 141–154. (doi:10.1016/S0739-7240(98)00002-2)

Malayer J, Hansen P, Gross T & Thatcher W 1990 Regulation of heat shock-induced alterations in the release of prostaglandins by the uterine endometrium of cows. *Theriogenology* **34** 219–230. (doi:10.1016/0093-691X(90)90516-V)

Menezo Y & Guerin P 1997 The mammalian oviduct: biochemistry and physiology. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* **73** 99–104. (doi:10.1016/S0301-2115(97)02729-2)

Miyamoto Y, Skarzynski D & Okuda K 2000 Is tumor necrosis factor α a trigger for the initiation of endometrial prostaglandin $F_{2\alpha}$ release at luteolysis in cattle? *Biology of Reproduction* **62** 1109–1115. (doi:10.1095/biolreprod62.5.1109)

Murakami M, Nakatani Y, Tanioka T & Kudo I 2002 Prostaglandin E synthase. *Prostaglandins and Other Lipid Mediators* **68–69** 383–399. (doi:10.1016/S0090-6980(02)00043-6)

Nabenishi H, Ohta H, Nishimoto T, Morita T, Ashizawa K & Tsuzuki Y 2011 Effect of the temperature-humidity index on body temperature and conception rate of lactating dairy cows in southwestern Japan. *Journal of Reproduction and Development* **57** 450–456. (doi:10.1262/jrd.10-135T)

Nishimura R, Komiyama J, Tasaki Y, Acosta T & Okuda K 2008 Hypoxia promotes luteal cell death in bovine corpus luteum. *Biology of Reproduction* **78** 529–536. (doi:10.1095/biolreprod.107.063370)

Okuda K, Kito S, Sumi N & Sato K 1988 A study of the central cavity in the bovine corpus luteum. *Veterinary Record* **123** 180–183. (doi:10.1136/vr.123.7.180)

Okuda K, Miyamoto Y & Skarzynski D 2002 Regulation of endometrial prostaglandin $F_{2\alpha}$ synthesis during luteolysis and early pregnancy in cattle. *Domestic Animal Endocrinology* **23** 255–264. (doi:10.1016/S0739-7240(02)00161-3)

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. *Scandinavian Journal of Clinical and Laboratory Investigation* **53** 757–763. (doi:10.3109/00365519309092582)

Pearl L & Prodromou C 2006 Structure and mechanism of the Hsp90 molecular chaperone machinery. *Annual Review of Biochemistry* **75** 271–294. (doi:10.1146/annurev.biochem.75.103004.142738)

Pennarossa G, Maffei S, Rahman M, Berruti G, Brevini T & Gandolfi F 2012 Characterization of the constitutive pig ovary heat shock chaperone machinery and its response to acute thermal stress or to seasonal variations. *Biology of Reproduction* **87** 119. (doi:10.1095/biolreprod.112.104018)

Putney D, Malayer J, Gross T, Thatcher W, Hansen P & Drost M 1988 Heat stress-induced alterations in the synthesis and secretion of proteins and prostaglandins by cultured bovine conceptuses and uterine endometrium. *Biology of Reproduction* **39** 717–728. (doi:10.1095/biolreprod39.3.717)

Sakatani M, Kobayashi S-I & Takahashi M 2004 Effects of heat shock on *in vitro* development and intracellular oxidative state of bovine preimplantation embryos. *Molecular Reproduction and Development* **67** 77–82. (doi:10.1002/mrd.20014)

Sakumoto R, Komatsu T, Kasuya E, Saito T & Okuda K 2006 Expression of mRNAs for interleukin-4, interleukin-6 and their receptors in porcine corpus luteum during the estrous cycle. *Domestic Animal Endocrinology* **31** 246–257. (doi:10.1016/j.domaniend.2005.11.001)

Siemieniuch M, Woclawek-Potocka I, Deptula K, Okuda K & Skarzynski D 2009 Effects of tumor necrosis factor-α and nitric oxide on prostaglandins secretion by the bovine oviduct differ in the isthmus and ampulla and depend on the phase of the estrous cycle. *Experimental Biology and Medicine* **234** 1056–1066. (doi:10.3181/0901-RM-23)

Suarez S 2008 Regulation of sperm storage and movement in the mammalian oviduct. *International Journal of Developmental Biology* **52** 455–462. (doi:10.1387/ijdb.072527ss)

Tanikawa M, Acosta T, Fukui T, Murakami S, Korzekwa A, Skarzynski D, Piotrowska K, Park C & Okuda K 2005 Regulation of prostaglandin synthesis by interleukin-1α in bovine endometrium during the estrous cycle. *Prostaglandins and Other Lipid Mediators* **78** 279–290. (doi:10.1016/j.prostaglandins.2005.09.003)

Tanikawa M, Lee H-Y, Watanabe K, Majewska M, Skarzynski D, Park S-B, Lee D-S, Park C-K, Acosta T & Okuda K 2008 Regulation of prostaglandin biosynthesis by interleukin-1 in cultured bovine endometrial cells. *Journal of Endocrinology* **199** 425–434. (doi:10.1677/JOE-08-0237)

Tanioka T, Nakatani Y, Kobayashi T, Tsujimoto M, Oh-ishi S, Murakami M & Kudo I 2003 Regulation of cytosolic prostaglandin E_2 synthase by 90-kDa heat shock protein. *Biochemical and Biophysical Research Communications* **303** 1018–1023. (doi:10.1016/S0006-291X(03)00470-4)

Uenoyama Y, Hattori S, Miyake M & Okuda K 1997 Up-regulation of oxytocin receptors in porcine endometrium by adenosine 3′,5′-monophosphate. *Biology of Reproduction* **57** 723–728. (doi:10.1095/biolreprod57.4.723)

Ulbrich S, Zitta K, Hiendleder S & Wolf E 2010 *In vitro* systems for intercepting early embryo-maternal cross-talk in the bovine oviduct. *Theriogenology* **73** 802–816. (doi:10.1016/j.theriogenology.2009.09.036)

Wijayagunawardane M, Miyamoto A, Cerbito W, Acosta T, Takagi M & Sato K 1998 Local distributions of oviductal estradiol, progesterone, prostaglandins, oxytocin and endothelin-1 in the cyclic cow. *Theriogenology* **49** 607–618. (doi:10.1016/S0093-691X(98)00011-9)

Wijayagunawardane M, Miyamoto A, Taquahashi Y, Gabler C, Acosta T, Nishimura M, Killian G & Sato K 2001 *In vitro* regulation of local secretion and contraction of the bovine oviduct: stimulation by luteinizing hormone, endothelin-1 and prostaglandins, and inhibition by oxytocin. *Journal of Endocrinology* **168** 117–130. (doi:10.1677/joe.0.1680117)

Received 27 November 2012
First decision 25 March 2013
Revised manuscript received 18 April 2013
Accepted 23 May 2013