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Spermatozoa stimulate prostaglandin synthesis and secretion in bovine oviductal epithelial cells

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Abstract

The dynamic action of oviductal secretory compounds on spermatozoa function is well documented. In contrast, the effect of spermatozoa on oviductal function remains poorly characterized. Previously, our lab and others have shown that prostaglandin (PG), together with other vasoactive peptides, plays major roles in oviductal contractions and sperm transport. We therefore examined the effect of spermatozoa on the production of PG by cow oviductal epithelial cells (OEC). A bovine spermatozoa–OEC co-culture system was utilized for this purpose. OECs in the second passage were co-cultured for 0, 1, 3, 6, 12, and 24 h with six doses of motile, killed, or truncated spermatozoa heads (control; without spermatozoa, 10^2–10^6 spermatozoa/ml medium). The levels of PGE_2 and $PGF_{2\alpha}$ in the medium were measured using enzyme immunoassays. Messenger RNA expression of cyclooxygenase-2, PGF synthase (PGFS), and PGE synthase (PGES) was investigated using real-time RT-PCR. The results indicated that motile spermatozoa increased the secretion of PG by OEC as well as cellular expression of mRNA for cyclooxygenase, PGES, and PGFS in a dose- and time-dependent manner. A maximum three- to fivefold increased secretion of PG was observed with a dose of 10^5 spermatozoa/ml after a 12-h co-incubation. Neither killed spermatozoa nor truncated spermatozoa heads stimulated oviductal biosynthesis and secretion of PG at any dose or time point observed. The results provide the first evidence that live spermatozoa in the oviduct up-regulate the local PG system, and thereby, enhance oviductal contractions. Thus, spermatozoa may bear a role in accelerating their own transport into the fertilization site.

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Introduction

The oviduct provides an optimal environment for the final maturation of spermatozoa, capacitation, acrosomal reaction as well as the survival of spermatozoa in the oviduct until the fertilization process. The dynamic action of oviductal secretions on spermatozoa survival and function during the long sojourn in the oviduct is well documented. Oviduct secretions (Parrish *et al.* 1989, Ellington 1991, McNutt and Killian 1991, Killian 2004), including specific glycoproteins (Abe *et al.* 1995), bicarbonate ions, progesterone (P_4), and oviductin (Boatman 1997), provide an optimal environment for spermatozoa in the oviduct. In addition, bovine oviductal epithelial cells (BOECs) also produce prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ that are reported to be actively involved in the regulation of oviductal contractions (Wijayagunawardane *et al.* 2001*b*). Our recent studies revealed that locally produced peptides such as endothelin-1 (ET-1), tumor necrosis factor (TNF)-α, angiotensin II (Ang II), antrial natriuretic peptide (ANP), and vascular endothelial growth factor (VEGF) stimulate PG secretion and oviductal motility during the periovulatory period (Wijayagunawardane *et al.* 2001*a*, 2001*b*, 2003, 2005, Wijayagunawardane & Miyamoto 2004*b*). These findings suggest that gamete transport in the cow oviduct is mediated by local vasoactive substances and cytokines, which reach their highest concentrations during the periovulatory stage.

Sperm–OEC interaction is highly specific, initiated by uncapacitated spermatozoa binding to OEC, and continued by the induction of capacitation (Fazeli *et al.* 1999). Bovine spermatozoa stimulate *de novo* protein synthesis in the bovine oviduct (Ellington *et al.* 1993), and in the mice oviduct, spermatozoa change the gene expression profiles (Fazeli *et al.* 2004) as well as the secretory proteome (Georgiou *et al.* 2005). However, the influence of spermatozoa directly on oviductal function remains poorly characterized. Thus, in the present study, we used capacitated spermatozoa and BOEC in a co-culture system to evaluate the effect of spermatozoa on the modulation of PG synthesis and secretion in the bovine oviduct.

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Materials and Methods

Isolation and culture of cow OEC

Oviducts from non-pregnant Holstein cows during the follicular phase were collected at a local slaughterhouse. The stage of the estrous cycle was identified according to the previous report (Wijayagunawardane *et al.* 1998) based on the morphology of the corpus luteum (CL), uterine fluid and cervical mucus characteristics, and luteal P_4 levels. The collected oviducts were transported to the laboratory in ice-cold Hanks' balanced salt solution (HBSS; Nissui pharmaceutical Co. Ltd, Tokyo, Japan).

The isolation and culture of BOEC were based on the method described previously (Wijayagunawardane *et al.* 1999*a*, 1999*b*). In brief, the surrounding connective tissues were trimmed from the oviduct, washed with HBSS, the oviductal lumen was flushed with 10 ml HBSS, and the BOEC were mechanically dislodged by squeezing the oviduct gently while flushing them with 15 ml HBSS. Then, the collected sheets of BOEC from several cows were pooled and washed twice with HBSS by centrifuging at 300 ***g*** for 10 min at 4 °C. Thereafter, the cell pellet was suspended in 4 ml HBSS, layered over 5 ml percol, and centrifuged at 900 ***g*** for 20 min at 4 °C. The cells in interphase were collected and washed twice with HBSS. The collected sheets of BOEC were cultured overnight in culture medium (Medium 199; HEPES modification; 0.85 g/l $NaHCO_3$, 60 mg/l penicillin, 100 mg/l streptomycin, and 56 mg/l ascorbic acid; Sigma Chemical Co.) supplemented with 5% fetal bovine serum (FBS; Bio Whittaker, Walkersville, MD, USA) at 38.5 °C in 5% CO_2 and 95% air. The BOECs in the suspension were taken, washed twice with HBSS, and trypsinized (0.05% trypsin–EDTA; Amresco, Salon, OH, USA) until single cells appeared. Subsequently, the BOECs were plated in six-well culture dishes (Nalge Nunc International, Roskilde, Denmark) and incubated in the culture medium supplemented with 5% FBS at 38.5 °C in 5% CO_2 and 95% air. After monolayer formation, cells were re-trypsinized and re-plated in six-well culture dishes (Nalge Nunc International) at a density of 3×10^4 ml and incubated at 38.5 °C in 5% CO_2 and 95% air in culture medium supplemented with 5% FBS, until growing BOEC monolayer was covered up to 70–80% of the bottom of the culture plate.

The average time taken for BOEC to form a monolayer was 5 days. The average cell concentration at the end of the experiment was 8×10^4 cells/ml. The characterization of bovine OEC was conducted based on a previous report (Rosselli *et al.* 1994). Briefly, cell viability was assessed in control and treated cells with Trypan blue staining and confirmed to be more than 90% viable at the time of plating, as well as at the end of the experiment. The purity of epithelial cell preparations was evaluated by reacting the cultured cells with monoclonal antibodies to cytokeratin (anti-cytokeratin, CK1) and immunostaining. The cells in culture showed characteristic epithelial morphology. Approximately 98% of the cells stained positively for CK1 antibodies.

Preparation of spermatozoa

Capacitation

Capacitation was achieved using Tyrode's albumin, lactate, and pyruvate medium (sp-TALP), as previously described (Parrish *et al.* 1988, 1989), with little modification. In brief, frozen thawed semen from five bulls were pooled and diluted with 5 ml conditioned sp-TALP. Following 1-h swim up at 38.5 °C and 5% CO_2 in air, the supernatant was recovered, pooled, and centrifuged at 350 ***g*** for 5 min. Then, the preparation was re-suspended in 1 ml sp-TALP and the concentration of the spermatozoa was calculated using a hemocytometer. Nigrosin–eosin staining was performed to evaluate the livability of spermatozoa, and more than 85% of live spermatozoa were obtained from the samples. Progressive motility was subjectively assessed by visual examination under a light microscope equipped with a stage warmer. These swim-up spermatozoa were used in the subsequent experiments.

Heat inactivation and decapitation of spermatozoa

Swim-up spermatozoa were subjected to heat inactivation in a water bath at 55 °C for 30 min to obtain dead spermatozoa. Immobilization was confirmed by subjective visual observation of samples under light microscope. Decapitation of spermatozoa was conducted as described previously (Kuretake *et al.* 1996, Tateno *et al.* 2000) with little modification. Briefly, a 15 ml Falcon tube (Nalge Nunc International) containing 7 ml swim-up spermatozoa suspension was placed on iced water, and the suspension was 'lightly' sonicated at 60% power output for 5 s using a 10 mm diameter horn (Sonics Vibra Cell, Sonics and Materials Inc., New town, CT, USA). The viability of decapitated spermatozoa was assessed by the eosin–nigrosin staining technique and confirmed to be more than 80%.

Experiment 1: dose-dependent effect of spermatozoa on release of PG from BOEC

Sub-confluent BOEC monolayers in the second passage were pre-incubated for 1 h in co-culture medium (TCM 199; HEPES modification; 0.85 g/l $NaHCO_3$, 60 mg/l penicillin, 100 mg/l streptomycin, and 56 mg/l ascorbic acid; Sigma Chemicals Co.) supplemented with 1% BSA, insulin (5 mg/l), transferrin (5 mg/l), and sodium selenite (5 μg/l). After pre-incubation, BOEC monolayers were co-cultured with four doses of (10^3–10^6 spermatozoa/ml) capacitated spermatozoa and 10^5 'dead' spermatozoa/ml for 12 h at 38.5 °C in 5% CO_2 in air ($n=7$ for each treatment). The BOEC monolayers

without spermatozoa and spermatozoa in co-culture medium without BOEC served as positive and negative controls respectively. An additional volume of sp-TALP was added to controls in order to control for any potential effect of the sp-TALP that was added with spermatozoa during the incubation.

Experiment 2: time-dependent effect of the spermatozoa on release of PG from BOEC

Based on the dose-dependent study (Experiment 1), a spermatozoa dose of 10^5 was used to evaluate the time-dependent effect of the spermatozoa on release of PG from BOEC. Sub-confluent BOEC monolayers in the second passage were pre-incubated in culture medium for 1 h, then co-cultured with 10^5 spermatozoa/ml for 1, 3, 12, and 24 h ($n=7$ for each treatment) at 38.5 °C in 5% CO_2 in air. The BOEC monolayer without spermatozoa served as the control.

Experiment 3: effect of the motile, dead, and decapitated spermatozoa on release of PG from BOEC

Sub-confluent BOEC monolayers in the second passage were pre-incubated in culture medium for 1 h and co-cultured with motile, dead, or decapitated spermatozoa at a dose of 10^5 spermatozoa/ml for 12 h ($n=7$ for each treatment) at 38.5 °C in 5% CO_2 in air. A BOEC monolayer without spermatozoa served as the control.

Cell preparation

After the desired period of incubation, the medium was collected from each well of six-well culture dishes and pooled. Cells in all six wells in a plate were trypsinized, washed twice with 15 ml 0.01 M PBS (PBS (−); pH 7.4; Sigma Chemicals Co.), and re-suspended in 6 ml PBS (−). A 50 μl portion of the cell suspension was used to evaluate the cell concentration. The remaining cells were again separated by centrifugation (300 ***g*** for 10 min at 4 °C), lysed with SV RNA Lysis Buffer (Promega Corporation), and stored at −80 °C until the RNA extraction.

Measurements of PG

The medium was centrifuged (300 ***g*** for 10 min at 4 °C) to remove any spermatozoa, and the subsequent sperm-free medium was used for performing enzyme immunoassays for PGE_2 and $PGF_{2\alpha}$.The assays were performed according to Miyamoto *et al.* (1995) and Wijayagunawardane *et al.* (1998) respectively. For PGE_2, within-assay and between-assay coefficients of variance (CV values) were 7.3 and 11.4% respectively. For $PGF_{2\alpha}$, within-assay and between-assay CV were 8.2 and 11.8%. The ED50 for the PGE_2 and $PGF_{2\alpha}$ assays were 260 and 355 pg/ml, and the ranges of the standard curves for these assays were 20–20 000 and 10–10 000 pg/ml respectively.

RNA extraction, cDNA synthesis, and real-time RT-PCR

RNA extraction from collected cells, cDNA synthesis, and real-time RT-PCR were conducted as previously described (Wijayagunawardane *et al.* 2005). Total RNA was extracted using the SV Total RNA Isolation System (Promega Corporation) according to the manufacturer's protocol. In the final step of SV Total RNA Isolation System, a DNase treatment was included to exclude residual genomic *DNA* or other contaminations. RNA concentration was measured using Bio-Tech Photometer (WPA, Cambridge, UK) at 260 nm absorbance and the concentration was adjusted to be 1 μg/μl. The extracted total RNA was stored in RNA storage solution (Ambion, Austin, Texas, USA) at −30 °C until used for cDNA synthesis.

First strand cDNA synthesis was conducted according to a commercial protocol described in SuperScript II Reverse Transcriptase (Invitrogen Corp). The first cocktail was prepared using 2 μg total RNA, 150 ng random primer (Invitrogen), 0.83 mM PCR Nucleotide Mix (dNTP; Roche Diagnostic Corp.), and H_2O, making the final volume of 12 μl/tube, subsequently incubated at 65 °C for 5 min in a thermal cycler (Mastercycler, Eppendorf, Netheler Hinz GmbH, Hamburg, Germany). The samples were kept on ice and 7 μl second cocktail; 0.01 M dithiothreitol (Invitrogen), 60 units of RNase inhibitor (RNasin, Promega), and First Strand Buffer (Invitrogen)/tube were added. Then, the samples were again incubated for 2 min at 42 °C and 200 units SuperScript II Reverse Transcriptase in 1 μl/tube were added. The thermal cycle program was run at 25 °C for 10 min, 42 °C for 50 min, and finally 75 °C for 15 min. The synthesized cDNA was stored at −30 °C.

The real-time RT-PCR (LightCycler; Roche diagnostics) and LightCycler-FastStart DNA Master SYBR Green 1 commercial kit (Roche) with commercially synthesized primers (Hokkaido System Science Co. Ltd, Sapporo, Japan; Table 1) were used to amplify each specific bovine transcript. Each capillary (Roche) contained 2 μl cDNA, 2 μl LightCycler-FastStart Reaction Mix SYBR Green 1 with LightCycler-FastStart Enzyme, 2.5 mM $MgCl_2$, 0.5 μM of each forward and reverse primers, and 14.8 μl H_2O; making the final volume of 20 μl/capillary. A negative control, which was prepared by replacing cDNA with PCR-grade water in the above mixture, was included in each run. The capillaries were centrifuged in LightCycler Carousal Centrifuge (Roche) before the amplification. The amplification was done with an initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 65 °C for 10 s, and the extension at 72 °C for 20 s. Finally, cooling was done at 30 °C. The melting curve was obtained from 70 to 95 °C at the rate of 0.1 °C/s.

For the quantification of each target gene, a DNA standard was constructed by amplifying a fragment of

Table 1 Primers used to amplify specific bovine transcripts.

Ligand	Primer	Sequence (position in cDNA)	Fragment size (bp)
COX-2	Forward	5′-TCCTGAAACCCACTCCCAACA-3′ (137–157)	242
	Reverse	5′-TGGGCAGTCATCAGGCACAG-3′ (359–379)	
PGES	Forward	5′-CGCTGCTGGTCATCAAAAT-3′ (73–90)	186
	Reverse	5′-GGAAGGGGTAGATGGTCTCC-3′ (238–259)	
PGFS	Forward	5′-GATGGCCACTTCATTCCTGT-3′ (34–53)	195
	Reverse	5′-CACAGTGCCATCTGCAATCT-3′ (208–229)	
β-actin	Forward	5′-CCAAGGCCAACCGTGAGAAGAT-3′ (374–395)	256
	Reverse	5′-CCACGTTCCGTGAGGATCTTCA-3′ (608–629)	

DNA (∼700 bp), which contained target sequence for quantitative PCR (100∼150 bp). The PCR products were subjected to electrophoresis; the target band was cut out and purified using a DNA purification kit (SUPRECTM-01; TaKaRa Bio Inc., Otsu, Japan). Three to five stepwise-diluted DNA standards were included in each PCR run. The quantification of mRNA expression was done using LightCycler Software (Version 3.5; Roche). The values were normalized using β-actin as the internal standard using delta–delta Ct method (Pfaffl 2001) and expressed as the percentage of control values.

Experimental design and statistical analysis

All experiments were carried out under the standard laboratory conditions with completely randomized design, keeping the controls for every treatment group concerned. The data on dose- and time-dependent effect of spermatozoa on PG release by BOEC as well as the effect of live, dead, and decapitated spermatozoa on PG biosynthesis and release were analyzed using one-way ANOVA followed by Tukey–Kramer test. Data on time-dependent effect of the PG production at each time point was separately analyzed using the simple paired *t*-test, followed by the *F*-test.

The mRNA expression values for cyclooxygenase-2 (COX-2), PGES, and PGFS were compared with the control values using ANOVA followed by the Tukey–Kramer test for the mean separation. The data were subjected to the arcsine transformation before the statistical analysis. $P<0.05$ were considered as significant.

Results

Within 1 h of co-incubation, almost all the surviving spermatozoa were observed to be bound to the BOEC monolayer. The spermatozoa that were bound to BOEC showed 80% viability after 24-h experimental period. The capacitated spermatozoa that were added to the medium without BOEC were observed to be dead after 3 h of incubation.

Experiment 1: effect of different doses of capacitated spermatozoa on the release of PG by BOEC monolayer

The spermatozoa dose dependently increased the release of both $PGF_{2\alpha}$ and PGE_2 from the BOEC (Fig. 1). When compared with controls, incubation of BOEC with spermatozoa 10^4, 10^5, and 10^6 ml for 12 h significantly increased ($P<0.05$–0.005) the PGE_2 release to the medium (Fig. 1a). Compared with the controls, 10^5 and 10^6 spermatozoa/ml showed a significant increase in the release of $PGF_{2\alpha}$ ($P<0.01$; Fig. 1b). Dead spermatozoa did not show any significant change in the release of PG. The intensity of PG release with 10^5 spermatozoa/ml was not significantly different from the dose of 10^6 spermatozoa/ml. In this series of

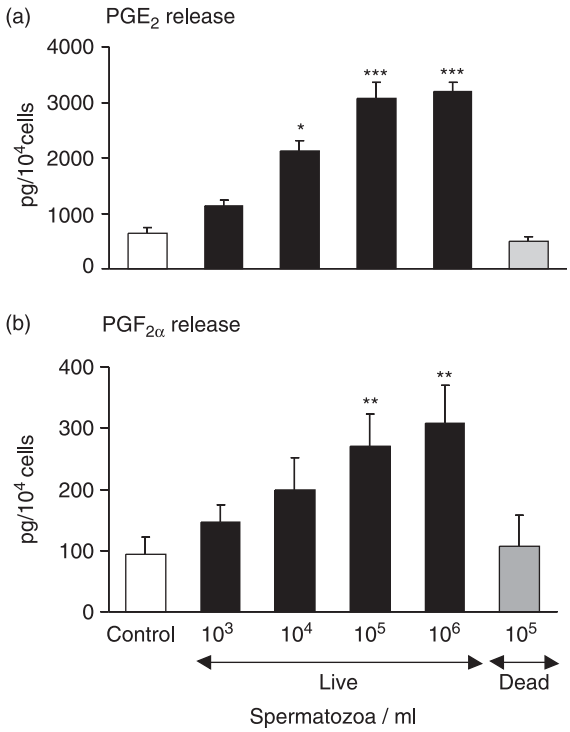

Figure 1 Dose-dependent response of capacitated spermatozoa on (a) PGE_2 and (b) $PGF_{2\alpha}$ release (mean±S.E.M.) by oviduct epithelial cell (BOEC) monolayers after 12 h of co-culture ($n=7$).*$P<0.05$, **$P<0.01$, **$P<0.005$.

experiments, 10^5 spermatozoa/ml was identified as the most effective dose for the PG release by BOEC.

Experiment 2: time-dependent effect of the PG release due to the stimulation of motile spermatozoa

Spermatozoa time dependently increased the release of both $PGF_{2\alpha}$ and PGE_2 from the BOEC (Fig. 2). The time-dependent data for PGE_2 showed that 6, 12 as well as 24-h co-incubation of spermatozoa with BOEC significantly stimulated ($P<0.01-0.005$) PGE_2 release, when compared with controls (Fig. 2a). When compared with 6-h co-incubation, 12- and 24-h co-incubation resulted in a higher ($P<0.05$) release of PGE_2 (Fig. 2b). However, the PGE_2 release between 12- and 24-h co-incubation was not significantly different. Only 12- and 24-h co-incubation showed a significant increase in $PGF_{2\alpha}$ release when compared with the control ($P<0.01-0.05$). There was no significant difference in the intensity of PG release between 12 and 24 h upon co-incubation. Therefore, 12-h incubation period was identified as the most effective time period of incubation for the PG release by BOEC. Dead spermatozoa did not show any significant change in the release of PG

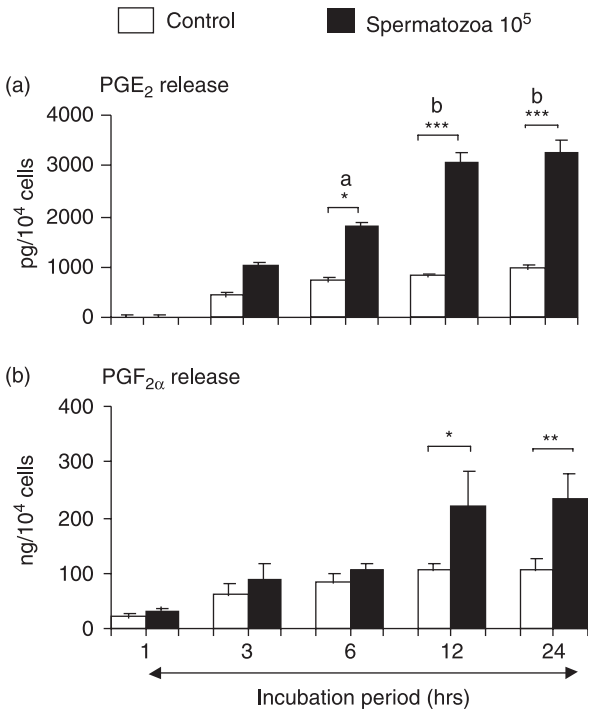

Figure 2 Time-dependent effect of capacitated spermatozoa (10^5 ml) on (a) PGE_2 and (b) $PGF_{2\alpha}$ release (mean ± S.E.M.) by bovine oviduct epithelial cell (BOEC) monolayers after 12 h of co-culture ($n=7$). *$P<0.05$, **$P<0.01$, ***$P<0.005$. [ab]Different letters denote a significant difference at $P<0.05$.

Experiment 3: PG release and mRNA expression in BOEC

Only live spermatozoa showed a significant effect on the release of PGs from BOEC, whereas dead or decapitated spermatozoa had no significant effect on the secretion of PG by BOEC. Capacitated live spermatozoa added to BOEC up-regulated ($P<0.01-0.005$) mRNA expression for COX-2, PGES as well as PGFS. Neither dead nor decapitated spermatozoa caused a significant level of mRNA expression for the COX-2 (Fig. 3a), PGES (Fig. 3b), and PGFS (Fig. 3c).

Discussion

The results of the present study provide the first direct evidence that live motile spermatozoa stimulate biosynthesis and secretion of PGE_2 and $PGF_{2\alpha}$ in the cow oviduct *in vitro*. These eicosanoids play a major role in oviductal motility by causing oviductal smooth muscle cells to contract and relax rhythmically, facilitating the spermatozoa transportation to the site of fertilization (Harper *et al.* 1980, Harper 1994). In the present study, only motile capacitated spermatozoa caused a significant increase in the expression of mRNA for COX-2, PGES, and PGFS along with the secretions of PGE_2 and $PGF_{2\alpha}$ by the BOEC. Dead or live spermatozoa heads did not show any effect on PG biosynthesis and secretion by BOEC. This phenomenon suggests that a mechanical stimulation of BOEC with the binding of motile spermatozoa may stimulate the PG system in the bovine oviduct. The ability of spermatozoa to induce gene expression in oviductal cells may involve a signal transduction mechanism, which is complex and involves several ligands from the sperm side that become activated after sperm binding to oviduct. We speculate that dead spermatozoa or sperm head alone are unable to present the appropriate ligands to achieve stimulation of oviductal cells.

In ruminants, $PGF_{2\alpha}$ and PGE_2 are the primary PGs produced in the female reproductive tract (Desnoyers *et al.* 1995), and in the cow oviduct, the levels of $PGF_{2\alpha}$ and PGE_2 were found to be higher during the periovulatory period of the estrus cycle (Wijayagunawardane *et al.* 1998). In the bovine endometrium, the PG synthesis is mainly governed by the rate-limiting enzyme COX-2, which converts arachidonic acid to the precursor PGH_2, where it is further converted to PGE_2 and $PGF_{2\alpha}$ by the key downstream enzymes of PGES and PGFS (Arosh *et al.* 2002, 2003, 2004*a*, 2004*b*). In the present study, it was observed that co-incubation of BOEC with spermatozoa significantly increased the mRNA expression for COX-2, PGES, and PGFS. This spermatozoa–oviductal interaction may be mediated by binding of the spermatozoa to the epithelium and at least, in part, due to the mechanical stimulation entrusted on the BOEC. Additionally, already identified

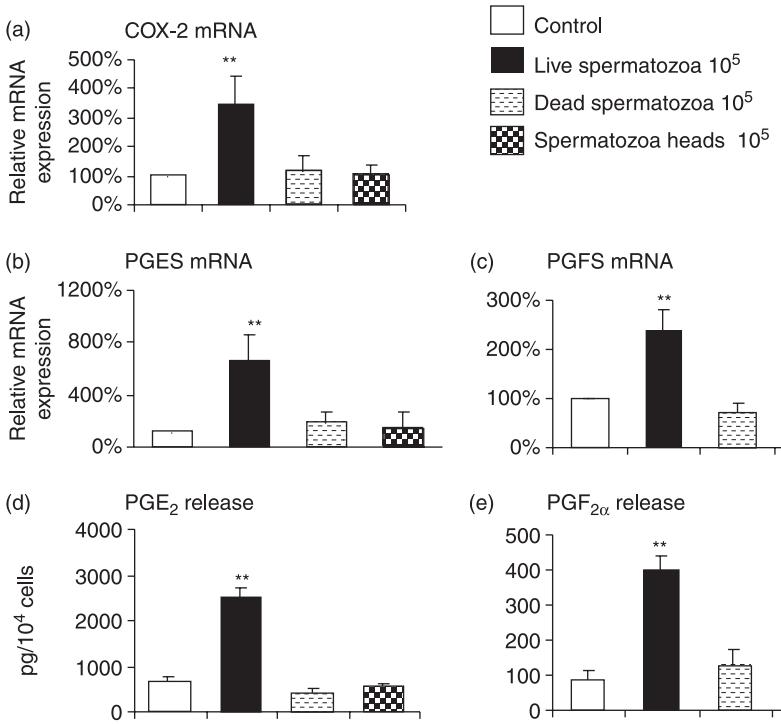

Figure 3 Effect of live, dead, and decapitated spermatozoa (10^5 ml) on the mRNA expression of (a) COX-2, (b) PGES, (c) PGFS in BOEC, (d) PGE_2 release, and (e) $PGF_{2\alpha}$ release by bovine oviduct epithelial cell (BOEC) at 12-h co-culture ($n=7$). $^{**}P<0.01$, $^{***}P<0.005$.

factors (Boilard *et al.* 2004, Ekhlasi-Hundrieser *et al.* 2005, Quintero *et al.* 2005), as well as numerous unidentified factors that may be secreted by live spermatozoa, can also be involved in this process (Quintero *et al.* 2005). However, a detail mechanism of cell-to-cell communication between spermatozoa and BOEC, which enhances biosynthesis and secretion of PG from the oviduct, awaits further investigation.

According to previous reports, capacitated spermatozoa have the ability to induce *de novo* protein synthesis and secretion within the cow oviduct, which creates an optimum microenvironment for the survival of gametes (Ellington *et al.* 1993). It was observed that the attachment of spermatozoa to the BOEC monolayer increased intracellular Ca^{++} levels, and this appears to enhance a cell-to-cell communication (Ellington *et al.* 1993). Therefore, calcium may be a potential candidate in mediating the cell-to-cell communication. A study on the mouse oviduct has revealed a system of spermatozoa recognition via a signal transduction pathway leading to a change in the oviductal gene expression profile (Fazeli *et al.* 2004).

Two separate studies on the gene expressions in the bovine oviduct using a combination of subtracted cDNA libraries and cDNA array hybridization (Bauersachs *et al.* 2003, 2004) give further evidence of complex mechanisms existing in the oviduct at molecular level, where alterations of differential gene expression in the different segments of the bovine oviduct directly alter the production of numerous cell-surface proteins, cell–cell interaction proteins, members of signal transduction pathways, immune-related proteins, and enzymes. These factors could also be the potential candidates involved in the spermatozoa–oviduct cross communication to enhance the biosynthesis of epithelial cell-borne bioactive substances in the bovine oviduct, and thus, would be a topic to be followed in future investigations.

Even though spermatozoa come into contact with cervical and uterine epithelia, the interaction with oviductal epithelium is unique due to the formation of oviductal–spermatozoa reservoir (Suarez *et al.* 1997). This reservoir acts to ensure the availability of sufficient viable spermatozoa for the capacitation and the survival of spermatozoa until fertilization (Lefebvre *et al.* 1997). In the present study, within 1 h of co-incubation, almost all the surviving spermatozoa were observed to be bound to the BOEC monolayer. Furthermore, the added dose of 10^4 spermatozoa/ml showed a significant effect on biosynthesis and secretion of PG. These observations suggest that spermatozoa physiologically or/and physically interact with oviductal epithelium to alter the local oviductal environment more favorable for its own function.

In our previous studies, we have shown that vasoactive peptides such as Ang II and ANP (Wijayagunawardane *et al.* 2001*a*), ET-1 (Wijayagunawardane & Miyamoto 2004*a*), together with VEGF (Wijayagunawardane *et al.* 2005) and LH (Wijayagunawardane *et al.* 2001*b*), stimulated PG secretion in the bovine oviduct during the periovulatory period. Moreover, the periovulatory LH surge with the basal level of P_4 from the regressing CL, together with the high level of E_2 from the Graafian follicle, have been shown to have a significant increase in PG release by the BOEC *in vitro* (Wijayagunawardane

et al. 2001*b*). Most importantly, PG, LH, ET-1 Ang II, and ANP directly stimulate the oviductal contraction (Wijayagunawardane *et al.* 2001*a*). In the present study, spermatozoa stimulate PG biosynthesis and secretion. Thus, during the periovulatory period, spermatozoa may stimulate oviductal motility through the elevation of local PG levels facilitating their timely transportation to the fertilization site. Interestingly, the maximum effective level of PG release was observed after 12 h of incubation, and this may be correlated with the timing of ovulation to assure the optimum number of spermatozoa at the site of fertilization.

In conclusion, the findings of the present study demonstrate for the first time that motile spermatozoa trigger PG biosynthesis and secretion in the cow oviduct. Elevated levels of PG may enhance oviductal motility to facilitate the timely transportation of spermatozoa to the site of fertilization.

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