***In vitro* regulation of local secretion and contraction of the bovine oviduct: stimulation by luteinizing hormone, endothelin-1 and prostaglandins, and inhibition by oxytocin**

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Abstract

The precise regulatory mechanisms of cyclic oviductal contraction in the cow are unclear. The purpose of this study was to evaluate the effect of luteinizing hormone (LH), steroids, prostaglandins (PGs) and peptides on the oviductal contraction and secretion of PGs and endothelin (ET-1). In addition, the cyclic expression of mRNA for ET-1 and its receptors (ET-R) was evaluated by reverse transcription-polymerase chain reaction (RT-PCR). In the *in vitro* microdialysis study, an infusion of LH alone or in combination with progesterone (P_4), estradiol-17 β (E_2) and/or ET-1 stimulated pronounced release of PGE₂, PGF_{2 α} and ET-1 in the oviducts from cows in the follicular and postovulatory phases. The addition of LH, LH+ P_4 + E_2 and/or ET-1 to the medium increased the amplitude of oviductal contraction. However, oxytocin (OT) completely blocked the responses of oviductal

secretion and contraction. In contrast, these substances did not show any effect in the oviducts from cows in the mid luteal phase. Similar expression patterns of mRNA encoding for ET-R type A and type B were found, which were highest during the postovulatory phase, lower during the luteal phase, with the lowest expression during the follicular phase. We suggest that the preovulatory LH surge, together with increasing E_2 levels from the Graafian follicle and a basal P_4 from regressing corpora lutea (CL), stimulates maximum oviductal production of PG and ET-1, resulting in oviductal contraction for a rapid transport of gametes. OT released from the newly-formed CL may block these mechanisms, and slow contractions for transport of the embryo to the uterus.

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Introduction

The oviduct plays a central role in the events of fertilisation and early embryonic development by providing an environment that facilitates sperm transport and capacitation, and oocyte transport and maturation. The simultaneous opposite movements of gametes to the fertilization site and the subsequent passage of the embryo into the uterus at the precise developmental stage are essential for successful pregnancy. Different contractile patterns of the oviduct occur during the periovulatory period for the movement of the zygote, which are strictly regulated by endocrine and paracrine/autocrine factors.

Studies on spontaneous oviduct contraction in the cow revealed low amplitude and frequency motility patterns during the luteal phase (Bennett *et al.* 1988), which

gradually increased with the drop in serum progesterone (P_4) prior to estrus (Ruckebusch & Bayard 1975). Both amplitude and frequency reach a maximum during estrus, but rapidly diminish over the next 3 days (Ruckebusch & Bayard 1975, Bennett *et al.* 1988). The acute withdrawal of endogenous estradiol-17 β (E_2) has been shown to be a potent stimulus to rabbit oviductal smooth muscle and contributes to an increase in muscular activity at ovulation (Halbert & Conrad 1975). During the retention of ova in porcine oviducts, the active preovulatory contractions disappeared, and ovum transport was arrested at the ampullar-isthmic junction (Rodriguez-Martinez *et al.* 1982b). In addition, an isthmic sphinctering effect controlling ovum transport through the oviduct has been reported (Lindblom *et al.* 1979). The motility pattern of sow oviduct during estrus was mostly antiperistaltic,

presumably related to sperm transport. However, during the periovulatory period the number of peristaltic and antiperistaltic waves became equal, perhaps in relation to the transport of gametes to the fertilization site (Rodriguez-Martinez *et al.* 1982a). These observations provide evidence that patterns of oviduct contraction during the periovulatory period are temporally and strictly controlled by local mechanisms that are responsive to the needs of the zygote.

The mechanisms involved in the regulation of contractions to facilitate gamete and embryo transport in the bovine oviduct are still unclear. The purpose of this study was, therefore, to examine the *in vitro* effect of luteinizing hormone (LH), oxytocin (OT) and ovarian steroids on oviduct contractions, as well as the secretion of prostaglandins (PG) and endothelin-1 (ET-1) by bovine oviduct epithelial cells. To investigate the secretions of the oviducts *in vitro*, we utilized a microdialysis system implanted in the lumen of oviducts that were maintained in organ culture chambers. In addition, the expression of mRNA for ET-1 and ET-1 receptors (ET-R) was evaluated in oviducts recovered during the follicular, postovulatory and luteal phases.

Materials and Methods

Source of the hormones

P₄, E₂, PGE₂ and PGF_{2α} were obtained from Sigma Chemical Co. (St Louis, MO, USA). OT and ET-1 were obtained from Peptide Institute Inc. (Osaka, Japan). LH (USDA-bLH-B-6) was a gift from the USDA Animal Hormone program (Baltimore, MD, USA).

Animals and sample collection

Reproductive tracts were collected from non-pregnant Holstein cows at a local slaughterhouse. The phase of the estrous cycle was determined according to the criteria of Ireland *et al.* (1980) by visual observation of the ovarian structures and the uterine mucous characteristics. The oviducts were classified as either follicular phase (days 18–20), postovulatory phase with a 3–5 mm diameter of point of rupture not covered by epithelium (days 1–2), or luteal phase (days 11–17). They were separated from the utero-tubal junction and surrounding connective tissues and trimmed. Oviducts for microdialysis experiments were transported to the laboratory in Medium 199 (M199; 25 mmol/l Hepes, 0.85 g/l NaHCO₃, 60 mg/l penicillin, 100 mg/l streptomycin, 56 mg/l ascorbic acid and 2 mg/l amphotericin B; Sigma Chemical Co.), which was maintained at 38 °C. Oviducts for the contraction experiments were transported in ice-cold Locke Ringer's solution (154 mmol/l NaCl, 5.6 mmol/l KCl₂, 2.4 mmol/l CaCl₂, 6.0 mmol/l NaHCO₃ and 5.6 mmol/l dextrose, pH 7.4).

For RNA extraction, the oviducts were frozen immediately in liquid nitrogen, transported to the laboratory and stored at –80 °C. The ampullary and isthmus regions of oviducts, both ipsilateral and contralateral to the functioning corpora lutea (CL) or developing follicle and ovulation site, were used in this study.

Estimation of the doses of the substances administered in microdialysis and contraction experiments

The concentrations of different substances used in this study were chosen based on measured concentrations in the bovine oviduct during the normal estrous cycle (Wijayagunawardane *et al.* 1996, 1998). Diffusion through the microdialysis capillary membrane was estimated as 1% for steroids and PG, and 0.1% for peptides (Miyamoto & Schams 1991, Miyamoto *et al.* 1997). Thus, the doses of steroid and PG were prepared so as to be 100 times greater than the concentrations estimated at the site of action, and the peptides doses were prepared so as to be 1000 times greater.

In vitro microdialysis system of bovine oviduct

The *in vitro* microdialysis system of the oviduct was adapted from the method described for the bovine CL (Miyamoto & Schams 1991). Briefly, the lumen of each 10 cm long oviductal segment was implanted with a 7 cm long and 0.2 mm diameter dialysis capillary membrane (Fresenius SPS 600 Hollow fiber, Fresenius AG, St Wendel, Germany: cut off 40 kDa) with each end glued to 5 cm long and 0.3 mm diameter silastic tubing. Both ends of the oviduct were then fixed to the silastic tubing by Histoacryl Blau (B Braun Melsungen AG, Melsungen, Germany) and incubated in M199 with 0.5% BSA (Sigma Chemical Co.) in simple organ culture chambers (modified 50 ml Falcon tubes; Becton Dickinson & Co, Lincoln Park, NJ, USA). In preliminary experiments, differences in response associated with side of ovulation or region of the oviduct were not observed. Therefore, each oviduct was divided into two segments, four oviductal segments from each cow were maintained in one organ culture chamber, and each segment was assigned randomly to different treatments as described below. Medium was continuously exchanged at a flow rate of 50 ml/h during the whole period of incubation at 38 °C. Both ends of the silastic tubing were connected to Teflon tubing and Ringer's solution was continuously perfused (1.3 ml/h) from one end using a peristaltic pump, while the other end was connected to a fraction collector. After a 2-h pre-incubation, the perfusate was collected in 4-h fractions for 16 h. The volume of a fraction was approximately 5.2 ml. Control (Ringer's solution only) and LH (1 µg/ml), P₄ (3.2 µmol/l), E₂ (370 nmol/l), OT (100 nmol/l), ET-1 (100 nmol/l), PGE₂ (1 µmol/l) or PGF_{2α} (100 nmol/l) in Ringer's solution were infused for

Figure 1 Position of the microdialysis membrane (arrow) in the oviduct. Magnification: left $\times 20$ and right $\times 200$.

4 h between 4 and 8 h of incubation. The same concentrations were also used in the consecutive microdialysis experiments; mixtures of treatment substances (control – Ringer's solution only, P_4+E_2 , $LH+E_2$, $LH+ET-1$, $LH+E_2+P_4$, $LH+ET-1+P_4$, $LH+E_2+ET-1+P_4$, $OT+LH$, $OT+LH+E_2$, $OT+E_2+P_4$, $OT+E_2+ET-1$ or $OT+LH+ET-1+E_2$) in Ringer's solution were infused for 4 h between 4 and 8 h of incubation. The 4-h fractions of perfusate were stored at -20°C until extraction for PG and peptides. After the experiment, the microdialysis capillary membranes implanted in the oviduct lumens were confirmed to be in tight contact with the surface of the epithelial cells (Fig. 1).

Hormone extraction

The fractions collected were warmed to room temperature, adjusted to pH 3.5 with 1 mol/l HCl and placed at room temperature for 1 h. PG and ET-1 extractions from microdialysis samples were performed as described earlier (Acosta *et al.* 1998). As a result of the extractions, PG and ET-1 were concentrated 12- and 52-fold respectively.

Samples were dissolved in assay buffers for enzyme immunoassays (EIAs) for PG (40 mmol/l PBS, 0.1% BSA, pH 7.2) and for peptide (42 mmol/l Na_2HPO_4 , 8 mmol/l KH_2PO_4 , 20 mmol/l NaCl, 4.8 mmol/l EDTA, 0.05% BSA, pH 7.5). The recovery rates, estimated by adding 3 different concentrations of PGE_2 (1, 0.5, 0.1 ng/ml), $\text{PGF}_{2\alpha}$ (1, 0.5, 0.1 ng/ml) and ET-1 (10, 5, 1 pg/ml) to the Ringer's solution were 78% for PGE_2 , 75% for $\text{PGF}_{2\alpha}$ and 63% for ET-1. The results presented are corrected for the differences in the recoveries of PG and ET-1.

Measurements of PG and ET-1

Concentrations of PGE_2 , $\text{PGF}_{2\alpha}$ and ET-1 in Ringer's extracts were measured using second antibody EIAs. The EIA for PGE_2 determination was performed as described previously (Wijayagunawardane *et al.* 1998). The method of EIA for $\text{PGF}_{2\alpha}$ is described elsewhere (Miyamoto *et al.* 1995). The ET-1 assay was performed as described earlier (Miyamoto *et al.* 1997). Within-assay and between-assay coefficients of variance were 7.3% and 11.4% for PGE_2 , 8.2% and 11.8% for $\text{PGF}_{2\alpha}$ and 9.6% and 13.2% for ET-1.

Table 1 Characterization of basal secretion of PGE₂, PGF_{2α} and ET-1 in the microdialysis system, and spontaneous contraction of bovine oviducts during different phases of the estrous cycle *in vitro*

Phases of the estrous cycle	Basal secretion (pmol/l Ringer's solution)			Spontaneous contraction	
	PGE ₂	PGF _{2α}	ET-1	Amplitude (gf)	Frequency (beats/min)
Follicular	1515 ± 596 ^a	1478 ± 516 ^a	2.9 ± 1.3 ^a	0.27 ± 0.07 ^a	3.45 ± 0.46 ^a
Postovulatory	1784 ± 437 ^a	1453 ± 550 ^a	3.5 ± 0.5 ^a	0.31 ± 0.06 ^a	3.49 ± 0.46 ^a
Luteal	349 ± 91 ^b	522 ± 85 ^b	1.4 ± 0.2 ^b	0.15 ± 0.09 ^b	1.90 ± 0.52 ^b

Means with different superscripts within a column are significantly different (*P*<0.05).

The ED₅₀s for the PGE₂, PGF_{2α} and ET-1 assays were 740 pmol/l, 1000 pmol/l and 180 pmol/l respectively, and the ranges of the standard curves for these assays were 85–40 000 pmol/l, 20–20 000 pmol/l and 4–4000 pmol/l respectively.

Measurement of oviduct contraction

One end of each of the 10 mm long oviduct segments was horizontally fixed through the lumen to a plastic holder, and the free end was attached to the arm of a force

displacement transducer (TB-625T; Nihon Koden, Tokyo, Japan) with a stainless steel hook and a silk ligature. A basal loading tension of 1 gf was maintained throughout the experiment. The preparation was soaked in Locke Ringer's solution in double-jacketed organ culture chambers (Iwaki Glass Co, Chiba, Japan) and maintained at 36 ± 0.5 °C. The temperature of the solution in the organ bath was held constant with an external water jacket and a thermoregulatory device (Thermominder Mini-80; Taiyo, Tokyo, Japan), and monitored with a thermistor (Model MAG-II; Shibaura Electric Co, Tokyo, Japan).

Table 2 Effects of the infusion of P₄, E₂, LH, ET-1, OT, PGE₂ and PGF_{2α} on the release of PGs and ET-1 in microdialyzed cow oviducts from the follicular phase of the estrous cycle (*n*=4, means ± S.E.M.; control=Ringer's solution only). The data are expressed as percentages of basal secretion (100%; 0–4 h)

Treatment		Incubation period (h)		
		4–8†	8–12	12–16
Secretion PGE ₂	Control	121 ± 48	111 ± 23	136 ± 25
	P ₄	173 ± 53	139 ± 38	133 ± 42
	E ₂	142 ± 33	181 ± 53	214 ± 54
	LH	428 ± 252	532 ± 118**	418 ± 128*
	ET-1	538 ± 151	338 ± 234	123 ± 92
	OT	232 ± 125	148 ± 53	134 ± 49
PGF _{2α}	Control	131 ± 34	152 ± 83	118 ± 28
	P ₄	348 ± 215	435 ± 142*	216 ± 79
	E ₂	732 ± 123**	548 ± 174**	635 ± 248*
	LH	638 ± 158**	734 ± 257*	664 ± 286
	ET-1	748 ± 138**	347 ± 223	432 ± 241
	OT	123 ± 48	154 ± 92	221 ± 98
ET-1	Control	121 ± 43	98 ± 22	148 ± 34
	P ₄	154 ± 81	193 ± 95	188 ± 67
	E ₂	138 ± 98	392 ± 116**	134 ± 24
	LH	519 ± 242	843 ± 98**	748 ± 223*
	OT	98 ± 42	115 ± 38	147 ± 45
	PGE ₂	118 ± 36	128 ± 82	146 ± 51
	PGF _{2α}	127 ± 27	154 ± 43	117 ± 48

†Indicates the period of treatments.
P*<0.05, *P*<0.01, significantly different from controls.

Table 3 Effects of combined treatment of hormonal stimulation on the release of PGs and ET-1 in microdialyzed cow oviducts collected from the follicular phase of the estrous cycle ($n=4$, means $\pm$ S.E.M.; control=Ringer's solution only). The data are expressed as percentages of basal secretion (100%; 0–4 h)

	Treatment	Incubation period (h)		
		4–8†	8–12	12–16
Secretion PGE ₂	Control	122 $\pm$ 32	101 $\pm$ 28	98 $\pm$ 31
	P ₄ +E ₂	147 $\pm$ 65	274 $\pm$ 78	197 $\pm$ 62
	LH+E ₂	458 $\pm$ 113*	724 $\pm$ 97*	543 $\pm$ 153*
	LH+ET-1	378 $\pm$ 86**	543 $\pm$ 124	254 $\pm$ 136
	LH+E ₂ +P ₄	452 $\pm$ 79***	875 $\pm$ 216*	724 $\pm$ 129**
	LH+ET-1+P ₄	652 $\pm$ 191*	912 $\pm$ 237*	789 $\pm$ 178*
	LH+ET-1+E ₂ +P ₄	824 $\pm$ 296*	1095 $\pm$ 317**	472 $\pm$ 382
	OT+LH	148 $\pm$ 22	204 $\pm$ 97	232 $\pm$ 123
	OT+LH+E ₂	125 $\pm$ 68	156 $\pm$ 67	148 $\pm$ 89
	OT+E ₂ +P ₄	214 $\pm$ 49	198 $\pm$ 78	157 $\pm$ 69
	OT+E ₂ +ET-1	227 $\pm$ 71	215 $\pm$ 40	196 $\pm$ 94
	OT+LH+ET-1	98 $\pm$ 67	214 $\pm$ 81	165 $\pm$ 54
	OT+LH+ET-1+E ₂	169 $\pm$ 72	137 $\pm$ 48	214 $\pm$ 57
PGF _{2α}	Control	118 $\pm$ 24	143 $\pm$ 38	127 $\pm$ 37
	P ₄ +E ₂	534 $\pm$ 127	297 $\pm$ 117	199 $\pm$ 84
	LH+E ₂	641 $\pm$ 158	547 $\pm$ 182*	817 $\pm$ 224**
	LH+ET-1	741 $\pm$ 217*	689 $\pm$ 117*	717 $\pm$ 173**
	LH+E ₂ +P ₄	856 $\pm$ 210**	759 $\pm$ 284**	987 $\pm$ 214***
	LH+ET-1+P ₄	824 $\pm$ 233*	1247 $\pm$ 311*	997 $\pm$ 484
	LH+ET-1+E ₂ +P ₄	1141 $\pm$ 228***	994 $\pm$ 324*	672 $\pm$ 157**
	OT+LH	240 $\pm$ 57	314 $\pm$ 137	97 $\pm$ 74
	OT+LH+E ₂	197 $\pm$ 67	257 $\pm$ 114	11 $\pm$ 967
	OT+E ₂ +P ₄	267 $\pm$ 132	158 $\pm$ 86	199 $\pm$ 69
	OT+E ₂ +ET-1	183 $\pm$ 81	234 $\pm$ 101	314 $\pm$ 145
	OT+LH+ET-1	218 $\pm$ 69	287 $\pm$ 137	217 $\pm$ 98
	OT+LH+ET-1+E ₂	193 $\pm$ 64	307 $\pm$ 137	267 $\pm$ 161
ET-1	Control	97 $\pm$ 26	154 $\pm$ 67	115 $\pm$ 29
	P ₄ +E ₂	351 $\pm$ 123	341 $\pm$ 184	210 $\pm$ 100
	LH+E ₂	489 $\pm$ 98**	628 $\pm$ 124*	348 $\pm$ 63**
	LH+E ₂ +P ₄	697 $\pm$ 139**	845 $\pm$ 214*	821 $\pm$ 121**
	OT+LH	167 $\pm$ 68	245 $\pm$ 117	197 $\pm$ 98
	OT+LH+E ₂	219 $\pm$ 91	197 $\pm$ 86	248 $\pm$ 196
	OT+E ₂ +P ₄	212 $\pm$ 87	162 $\pm$ 67	154 $\pm$ 48

†Indicates the period of treatments.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, significantly different from controls.

The solution was bubbled with a mixture of 95% O₂ and 5% CO₂ and exchanged at a rate of 60 ml/h using a peristaltic pump. The signals were amplified by an isometric amplifier (RAT-1200 M; Nihon Koden), digitized with an analog-to-digital converter (MacLab/4S; AD Instruments Pty Ltd, Castle Hill, Australia) and stored on a hard disk of a personal computer (Macintosh Power book 190cs; Apple Computer Inc., Cupertino, CA, USA).

The preparation was equilibrated in a bathing solution for 2 h and then data were recorded for 7 h. After the start of the 7-h experimental period, different hormones were added to the Locke Ringer's solution. The hormones were LH (100 ng/ml), ET-1 (10 nmol/l), OT (10 nmol/l), PGE₂ (100 nmol/l), PGF_{2 α} (10 nmol/l), P₄ (32 nmol/l)

and E₂ (37 nmol/l). Because the actions of different hormones on the oviduct contraction were not influenced by side of ovulation or region of the oviduct, the oviduct segments obtained from one cow were randomly allocated for different combinations of treatments (LH+ET-1, LH+OT, LH+ET-1+E₂+P₄, or LH+ET-1+E₂+P₄+OT).

RNA extraction

Total RNA was extracted from the oviduct (2 cm of the whole isthmus) following the protocol of Chomczynski & Sacchi (1987) using Trizol reagent (Gibco BRL, Gaithersburg, MD, USA). RNA concentration of each

Table 4 Effects of the infusion of P₄, E₂, LH, ET-1, OT, PGE₂ and PGF_{2α} on the release of PGs and ET-1 in microdialyzed cow oviducts from the postovulatory phase of the estrous cycle (n=8–10, means ± S.E.M.; control=Ringer's solution only). The data are expressed as percentages of basal secretion (100%; 0–4 h)

		Incubation period (h)		
		4–8†	8–12	12–16
Secretion PGE ₂	Control	101 ± 33	161 ± 47	116 ± 15
	P ₄	278 ± 71	190 ± 58	157 ± 53
	E ₂	387 ± 61	292 ± 91	269 ± 126
	LH	313 ± 81	864 ± 532*	660 ± 380*
	ET-1	300 ± 51	418 ± 111	196 ± 85
	OT	334 ± 96	268 ± 134	112 ± 39
PGF _{2α}	Control	78 ± 14	80 ± 13	71 ± 22
	P ₄	577 ± 138	867 ± 295	516 ± 149
	E ₂	1015 ± 365**	820 ± 417	599 ± 338
	LH	821 ± 240*	1337 ± 460	933 ± 339*
	ET-1	508 ± 79*	1287 ± 318**	671 ± 141*
	OT	238 ± 23	243 ± 126	180 ± 73
ET-1	Control	85 ± 13	91 ± 11	94 ± 14
	P ₄	224 ± 81	233 ± 82	193 ± 44
	E ₂	291 ± 42	446 ± 165*	93 ± 36
	LH	543 ± 123***	609 ± 119**	748 ± 223***
	OT	291 ± 33	209 ± 39	146 ± 32
	PGE ₂	109 ± 22	158 ± 94	107 ± 18
	PGF _{2α}	93 ± 14	89 ± 28	154 ± 79

†Indicates the period of treatments.
*P<0.05, **P<0.01, ***P<0.001, significantly different from controls.

sample was determined by UV-spectroscopy (OD 260 nm). Integrity of total RNA was controlled by agarose/formaldehyde gel electrophoresis followed by ethidium bromide staining.

Reverse transcription-polymerase chain reaction (RT-PCR)

Four micrograms total RNA were subjected to first-strand cDNA synthesis using 2.5 μM random hexamers (Pharmacia, Freiburg, Germany) and the Superscript II reverse transcriptase (Gibco BRL) in a final volume of 60 μl as previously described (Gabler *et al.* 1998). The following commercially synthesized primers (Gibco BRL) were used to amplify specific bovine transcripts: ET-1 (333 bp) forward: 5' TCC CCA GAA TGG ATT ATT TCC 3', reverse: 5' TCT TCC TGT GGA CTG TCG C 3'; ET-R type A (329 bp) forward: 5' TGC AGA AGT CCT CAG TGG G 3', reverse: 5' GAT CGC AGT GCA CAC CAG 3'; ET-R type B (296 bp) forward: 5' AAA CTG AGA ATC TGC TTG CTC C 3', reverse: 5' AGA GTG AGC TTC AAA ATC CTG C 3'. The predicted size of each RT-PCR product is shown in parentheses.

Aliquots of cDNA (5 μl) were amplified with 0.5 U Taq polymerase (QIAGEN, Valencia, CA, USA) in a thermal Cycler (Biometra, Goettingen, Germany) in a final volume of 25 μl, as previously described (Gabler *et al.* 1997). The following amplification programs were applied for

ET-1 (40 cycles at 94 °C and 60 °C, 1 min each), as well as 29 cycles for ET-R type A and 31 cycles for ET-R type B (94 °C and 60 °C, 1 min each) respectively. An 18S rRNA-PCR (324 bp) was performed to control the integrity of the RNA, as well as the efficiency of the reverse transcription for each sample. The 18S primers and the appropriate competitors were used in a ratio of 2:3 (Ambion, Austin, TX, USA). Twenty-four amplification cycles were performed (94 °C, 60 °C and 72 °C, 30 s each). As a negative control, water was used instead of RNA for the RT-PCR to exclude any contamination from buffers and tubes. Furthermore, an additional control was performed omitting the reverse transcriptase to exclude any amplification of genomic DNA.

Aliquots of 5 μl per reaction were run on 1.5% (w/v) agarose gels containing 1.0 μg/ml ethidium bromide. To confirm the identity of the PCR products, each PCR product was sequenced. The resultant band intensities were scanned by a video documentation system (Eagle Eye, Stratagene, La Jolla, CA, USA) and analyzed with the Gel-Pro Analyzer software (Media Cybernetics, Silver Spring, MD, USA).

Statistical analysis

The mean concentrations of each of the substances in the first 4 h of incubation (perfusion with Ringer's solution

Table 5 Effects of combined treatment of hormonal stimulation on the release of PGs and ET-1 in microdialyzed cow oviducts collected from the postovulatory phase of the estrous cycle ($n=4-5$, means $\pm$ S.E.M.; control=Ringer's solution only). The data are expressed as percentages of basal secretion (100%; 0–4 h)

	Treatment	Incubation period (h)		
		4–8†	8–12	12–16
Secretion PGE ₂	Control	98 $\pm$ 20	89 $\pm$ 21	100 $\pm$ 21
	P ₄ +E ₂	324 $\pm$ 82	464 $\pm$ 88	285 $\pm$ 58
	LH+E ₂	1243 $\pm$ 495***	1006 $\pm$ 397*	968 $\pm$ 533*
	LH+ET-1	269 $\pm$ 57	909 $\pm$ 475**	930 $\pm$ 201***
	LH+E ₂ +P ₄	267 $\pm$ 28	515 $\pm$ 119**	368 $\pm$ 122
	LH+ET-1+P ₄	265 $\pm$ 185	873 $\pm$ 249**	584 $\pm$ 158*
	LH+ET-1+E ₂ +P ₄	401 $\pm$ 69*	907 $\pm$ 103**	890 $\pm$ 282***
	OT+LH	207 $\pm$ 37	164 $\pm$ 61	122 $\pm$ 41
	OT+LH+E ₂	276 $\pm$ 54	363 $\pm$ 133	250 $\pm$ 49
	OT+E ₂ +P ₄	125 $\pm$ 28	249 $\pm$ 53	164 $\pm$ 47
	OT+E ₂ +ET-1	176 $\pm$ 44	148 $\pm$ 22	96 $\pm$ 20
	OT+LH+ET-1	142 $\pm$ 66	146 $\pm$ 38	86 $\pm$ 20
	OT+LH+ET-1+E ₂	211 $\pm$ 44	234 $\pm$ 21	122 $\pm$ 20
	PGF _{2α}			
	Control	122 $\pm$ 12	114 $\pm$ 15	117 $\pm$ 36
PGF _{2α}	P ₄ +E ₂	910 $\pm$ 270**	838 $\pm$ 348*	898 $\pm$ 363*
	LH+E ₂	1438 $\pm$ 358	949 $\pm$ 282*	613 $\pm$ 204*
	LH+ET-1	956 $\pm$ 320*	1431 $\pm$ 517*	2075 $\pm$ 970*
	LH+E ₂ +P ₄	1351 $\pm$ 402**	1197 $\pm$ 433*	2003 $\pm$ 693**
	LH+ET-1+P ₄	801 $\pm$ 233*	2002 $\pm$ 458***	609 $\pm$ 201
	LH+ET-1+E ₂ +P ₄	1225 $\pm$ 133**	2269 $\pm$ 424***	1233 $\pm$ 742
	OT+LH	568 $\pm$ 240	771 $\pm$ 199	560 $\pm$ 143
	OT+LH+E ₂	402 $\pm$ 374	469 $\pm$ 359	550 $\pm$ 378
	OT+E ₂ +P ₄	253 $\pm$ 123	322 $\pm$ 184	143 $\pm$ 20
	OT+E ₂ +ET-1	249 $\pm$ 82	324 $\pm$ 192	211 $\pm$ 93
	OT+LH+ET-1	139 $\pm$ 12	156 $\pm$ 37	159 $\pm$ 62
	OT+LH+ET-1+E ₂	420 $\pm$ 417	577 $\pm$ 349	266 $\pm$ 183
	ET-1			
	Control	102 $\pm$ 16	99 $\pm$ 59	74 $\pm$ 23
	P ₄ +E ₂	492 $\pm$ 116**	491 $\pm$ 169*	433 $\pm$ 105*
	LH+E ₂	553 $\pm$ 121***	455 $\pm$ 96**	248 $\pm$ 47
	LH+E ₂ +P ₄	244 $\pm$ 31**	398 $\pm$ 41**	301 $\pm$ 132
	OT+LH	66 $\pm$ 27	108 $\pm$ 28	67 $\pm$ 27
	OT+LH+E ₂	123 $\pm$ 23	81 $\pm$ 37	97 $\pm$ 58
	OT+E ₂ +P ₄	80 $\pm$ 25	79 $\pm$ 28	205 $\pm$ 94

†Indicates the period of treatments.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, significantly different from controls.

only) were used to calculate the individual baseline for each hormone, because of a large variation in the absolute amount of hormones released into each of the microdialysis capillary membranes implanted in the various oviducts. All values were expressed as a percentage of the corresponding baseline. The coefficient of variation in the absolute concentration of each hormone in the microdialysis perfusates collected from different oviducts during the first 4 h (baseline) was as follows: PGE₂=84–304%, PGF_{2 α} =77–269%, and ET-1=41–73%. This transformation enables an evaluation of relative changes of hormonal values between different oviducts. The effects of the infused substances on the release of PGE₂, PGF_{2 α} , and ET-1 were compared with control values obtained during the same time

period using ANOVA followed by Duncan's new multiple range test. Differences were considered significant at a probability less than 5% ($P<0.05$).

Amplitude and frequency of oviduct contractions in the control and treatment groups were averaged over 10-min intervals and analyzed using the MacLab Chat data acquisition/analysis program (1997; MacLab, AD Instruments). The basal oviduct amplitude varied considerably among individual oviducts. Therefore, the baseline of oviduct amplitude (defined as 100%) was calculated by averaging the data for the first hour, and the amplitude of each 10-min interval was then expressed as a percentage of this individual baseline. As the basal amplitude was very stable, the effects of the added substances on the

Table 6 Effects of the infusion of LH, ET-1, OT, LH+OT and LH+ET-1+OT in microdialyzed cow oviducts from the luteal phase of the estrous cycle (*n*=4–5, means ± S.E.M.; control=Ringer’s solution only). The data are expressed as percentages of basal secretion (100%; 0–4 h)

		Incubation period (h)		
		4–8†	8–12	12–16
Secretion				
	PGE ₂			
	Control	122 ± 21	192 ± 52	122 ± 52
	LH	183 ± 29	270 ± 63	271 ± 23
	ET-1	252 ± 68	201 ± 37	214 ± 85
	OT	191 ± 33	256 ± 113	133 ± 33
	LH+ET-1	165 ± 15	216 ± 49	131 ± 20
PGF _{2α}	LH+OT	235 ± 59	322 ± 48	190 ± 29
	LH+ET-1+OT	467 ± 162*	426 ± 162	509 ± 164*
	Control	102 ± 32	93 ± 10	113 ± 16
	LH	376 ± 238	186 ± 69	377 ± 196
	ET-1	416 ± 95	203 ± 60	352 ± 64
	OT	230 ± 71	391 ± 98	155 ± 40
	LH+ET-1	396 ± 223	305 ± 149	309 ± 113
ET-1	LH+OT	64 ± 16	157 ± 44	194 ± 71
	LH+ET-1+OT	169 ± 90	279 ± 66	252 ± 100
	Control	57 ± 28	144 ± 42	97 ± 50
	LH	153 ± 25*	221 ± 65	223 ± 62
	OT	130 ± 48	267 ± 100	208 ± 95
	LH+OT	110 ± 7	193 ± 73	145 ± 57

†Indicates the period of treatments.
**P*<0.05, significantly different from controls.

contraction were compared with control values obtained during the same time point using ANOVA followed by Duncan’s new multiple range test.

Statistical analyses of ET-1, ET-R A and ET-R B mRNA were corrected with the signal intensities of the 18S rRNA. The expression of ET-1, ET-R A and ET-R B during different phases of the estrous cycle was analyzed using ANOVA. When ANOVA showed significant differences, the Tukey–Kramer test was used to test significance. Probabilities less than 0.05 (*P*<0.05) were considered significant.

Results

The basal release of substances in the microdialysis system, as well as the spontaneous amplitude and frequency of the oviduct contraction in the control group were constant and stable during the experimental periods. The basal release of all substances measured and the spontaneous amplitude and frequency of the oviduct contractions were higher (*P*<0.05) during the follicular and postovulatory phases than during the luteal phase (Table 1). Moreover, the contraction pattern of the oviducts was more regular during the follicular and postovulatory phases than during the luteal phase.

Effect of substance infusion on PG and ET-1 secretion from microdialyzed oviduct from the follicular and postovulatory phases

E₂ stimulated PGF_{2α} release (4–16 h; *P*<0.05 to *P*<0.01) and ET-1 release (8–12 h; *P*<0.01) in follicular phase oviducts, while P₄ only stimulated PGF_{2α} secretion (8–12 h; *P*<0.05; Table 2). ET-1 stimulated PGF_{2α} release (*P*<0.01) during infusion, although PG showed no effect on the ET-1 secretion. LH stimulated PG and ET-1 secretion (*P*<0.05 to *P*<0.01), and OT had no effect on PG or ET-1 release (Table 2). The infusion of LH with different combinations of P₄, E₂ and/or ET-1 resulted in an acute and continuous increase in the release of PG and ET-1 (*P*<0.05 to *P*<0.001). However, simultaneous administration of OT with different combinations of LH, P₄, E₂ and ET-1 did not alter the basal PG or ET-1 release (Table 3).

A 4-h infusion of E₂ induced an acute PGF_{2α} release (*P*<0.01), and E₂ stimulated ET-1 release 8–12 h (*P*<0.05) in postovulatory phase oviducts (Table 4). The ET-1 infusion stimulated PGF_{2α} release in the oviducts from the postovulatory phase (*P*<0.05 to *P*<0.01). LH stimulated PG secretion (*P*<0.05) and ET-1 secretion (*P*<0.01 to *P*<0.001), which was sustained throughout the experimental period (Table 4). The combined infusion of P₄ and E₂ significantly (*P*<0.05 to *P*<0.01) increased

Figure 2 Effect of different substances on the amplitude (bars) and frequency (line) of the contractions of the oviducts collected during the follicular phase ($n=4-5$, means $\pm$ S.E.M.). Solid bars indicate the period of treatment. * $P<0.05$, significantly different from controls at the same time point.

PGF_{2α} and ET-1 production ($P<0.05$ to 0.01 ; Table 5). Combined infusion of LH with P₄, E₂ and/or ET-1 resulted in an increase in PG and ET-1 release ($P<0.05$ to $P<0.001$). However, simultaneous administration of OT with different combinations of LH, P₄, E₂ and ET-1 abolished stimulation of PG and ET-1 release (Table 5).

Effect of substance infusion on PG and ET-1 secretion from microdialyzed oviduct from the luteal phase

The basal release of PG and ET-1 during the luteal phase was significantly lower ($P<0.05$) than that of the follicular and postovulatory phases (Table 1). Although some substances stimulated the production of PGE₂, PGF_{2α} and ET-1, the magnitude of this stimulation was significantly lower ($P<0.05$) than that observed during the follicular or postovulatory phases (Table 6).

Effect of substances on the oviduct contraction in vitro

Administration of LH, ET-1, PGE₂, PGF_{2α}, LH+ET-1 and LH+ET-1+P₄+E₂ significantly increased ($P<0.05$) the amplitude of oviduct contraction in both follicular and postovulatory phase oviducts. The stimulation of the amplitude of oviduct contraction by the combination of LH+ET-1+P₄+E₂ during the postovulatory and follicular phases, as well as the stimulation by LH during the

follicular phase persisted up to 4 h after the withdrawal of treatment. However, stimulation by other substances or combinations lasted for 1–2 h after the treatments. The combination of OT with other substances completely blocked contractile amplitude. None of these treatments altered the basal frequency of the oviduct contraction during any phase of the estrous cycle (Figs 2 and 3). Moreover, in luteal phase oviducts, the above treatments did not affect the amplitude or frequency of contractions (Fig. 4).

Expression of mRNA for ET-1 and ET-R types A and B

Specific transcripts for ET-1, ET-R A and ET-R B were detected in the bovine oviduct. Each PCR product showed 100% homology to the known bovine genes after sequencing. Quantity and quality of each RNA sample were verified after denaturing agarose gel electrophoresis, as well as comparative RT-PCR for the ribosomal 18S rRNA (Fig. 5). The relative signal intensities for PCR products specific for ET-1, ET-R A and ET-R B were assessed after correction based on the 18S rRNA signal intensities. The same expression pattern for both endothelin receptors, as well as for the ligand endothelin was found during the estrous cycle: highest expression during the phase after ovulation, lower expression during the luteal phase and the lowest expression before ovulation (Fig. 6).

Figure 3 Effect of different substances on the amplitude (bars) and frequency (line) of the contractions of the oviducts collected during the postovulatory phase. ($n=4-5$, means $\pm$ S.E.M.) Solid bars indicate the period of treatment. * $P<0.05$, significantly different from controls at the same time point.

This expression pattern was significant for ET and ET-R B as shown by ANOVA. The difference of the ET-R A expression during the postovulatory and follicular phases approached significance ($P=0.0642$).

Discussion

The results in this study indicate that LH enhances the *in vitro* secretion of PGE_2 , $PGF_{2\alpha}$ and ET-1, as well as the contractile amplitude of bovine oviducts from the follicular and postovulatory periods, but not those from the luteal period. Furthermore, ET-1 stimulated the release of PGE_2 and $PGF_{2\alpha}$ from the oviduct epithelial cells in the microdialysis experiment. Our recent observations of the cyclic changes in local content of PG and ET-1 in the bovine oviduct revealed that the oviduct contains the highest concentrations of PG and ET-1 during the periovulatory period (Wijayagunawardane *et al.* 1998). This was confirmed by the present results in which the secreted levels of PG and ET-1 were higher during the follicular and postovulatory periods than during the luteal phase in the microdialysis study. Furthermore, we found that ET-R A and B mRNAs were highly expressed after ovulation, suggesting that the functional ET system is primarily active after ovulation *in vivo*.

The demonstration of LH/human chorionic gonadotropin (hCG) receptors in the oviduct of woman (Lei *et al.* 1993, Han *et al.* 1996), sow (Derecka *et al.* 1995) and cow (Sun *et al.* 1997) suggested a direct involvement of LH/hCG in early pregnancy events (Lei *et al.* 1993, Derecka *et al.* 1995, Han *et al.* 1996). It was reported that LH increased 5-lipoxygenase, cyclooxygenase (COX)-1 and COX-2 enzymes in the human oviduct (Lei *et al.* 1993, Han *et al.* 1996), and increased PG release in human umbilical cord (Rao *et al.* 1993), cultured endothelial cells (Shemesh *et al.* 1997), and human fetal membranes (Toth *et al.* 1996). Moreover, LH activates protein kinase (PK) A and PKC in porcine myometrial smooth muscle cells (Kisielewska *et al.* 1996) and such an activation of PK results in increased ET-1 mRNA levels in cultured bovine endothelial cells (Emori *et al.* 1991). The results of the present study clearly indicated that LH stimulates the secretion of PGE_2 , $PGF_{2\alpha}$ and ET-1 in the oviduct, and that LH also increases oviduct contraction. We postulate that the LH-induced oviduct contraction, at least in part, is due to the increased secretion of PGE_2 , $PGF_{2\alpha}$ and ET-1.

PGs are reported to modulate oviduct contraction, through effects of E-series PG on relaxation of smooth muscle and of F-series PG on contraction (Spilman & Harper 1975). Human oviduct musculature responds to

Figure 4 Effect of different substances on the amplitude (bars) and frequency (line) of the contractions of the oviducts collected during the luteal phase ($n=4-5$, means $\pm$ S.E.M.). Solid bars indicate the period of treatment.

PGF_{2α} by increasing tonus (Atanackovic *et al.* 1983), and PGF_{2α} is recognized as a prerequisite for maintenance of normal tubal contractions (Lindblom & Andersson 1985). The PGF_{2α} receptors in the adult rat oviduct predominated during the periovulatory period (Orlicky & Williams-Skipp 1993) and the response of the oviduct to PGF_{2α} increased markedly following ovulation in marmoset monkeys (Barbosa *et al.* 1980). Also, PGE₂ treatment hastened the oviduct transport of equine embryos (Weber *et al.* 1991). The direct stimulation of the amplitude of oviduct contraction by PG during the follicular and postovulatory phases observed in this study may be due to the increased sensitivity as a result of an increase in the number of binding sites during this period.

ET-1-induced contractile responses and increased oscillation frequency in rat uterine smooth muscle appear to be mediated through ET-R type A (Tsunoda *et al.* 1993). Rosselli *et al.* (1994) observed ET-1 production by bovine oviduct epithelial cells and hypothesized the possible involvement of ET-1 in the muscular oviduct wall contraction. Moreover, ET-1 acts in bovine oviduct arteries by directly activating a homogenous population of ET-R type A in smooth muscle (Labadia *et al.* 1997). In the present study, oviduct expression of mRNA for ET-R, both A and B types, showed a similar pattern with the highest mRNA levels observed during the postovulatory phase. Smooth muscle cell is the predominant cell type in

the oviduct isthmus and both ET receptors are believed to be primarily expressed in smooth muscle cells. Because the maximum stimulatory effect of ET-1 was observed during the postovulatory phase in the present study, it may be the result of elevated ET-R concentration in the oviduct smooth muscles during this period. It was reported that ovarian steroids play a role in the control of expression of ET-R in human endometrium (Collett *et al.* 1996) and that endogenous E₂ increases the affinity of high-affinity ET-R in coronary arterial smooth muscle of female pigs (Barber *et al.* 1996). Moreover, we recently reported that the local distribution of E₂ in the cow oviduct is highest during the follicular phase (Wijayagunawardane *et al.* 1998). Therefore, the stimulatory effect of ET-1 on oviduct secretion and contraction during the follicular phase, even with the lowest ET-R levels in the oviduct, may be due to increases in the affinity of the ET-R by the elevated local levels of E₂ in the oviduct.

Surprisingly, OT completely blocked increases in secretion of PG and ET-1 and increases in the contractile amplitude that were stimulated by different combinations of substances used in the present study. This is the first evidence that OT may act locally on oviduct function as a suppressor. In sheep OT is locally recirculated to the oviduct in a higher concentration than is supplied by peripheral blood (Schramm *et al.* 1986) and in women local oviductal tissue levels of OT exceeded its plasma

Stage of the estrous cycle

Figure 5 Specific RT-PCR products for ET-1 (333 bp), ET-R A (329 bp), ET-R B (296 bp) and 18S rRNA (324 bp) separated by agarose gel electrophoresis. One representative result is shown for each estrous cycle phase (n=4).

concentrations (Lundin *et al.* 1989). OT increased the contractile activities in the cow oviduct isthmus (Ruckebusch & Bayard 1975, Bennett *et al.* 1988), and oviduct musculature was most sensitive to physiological concentrations of OT at estrus in the ewe (Gilbert *et al.* 1992). Furthermore, the ovine oviduct contains more OT receptors at the postovulatory phase than during the luteal phase (Ayad *et al.* 1991). These observations suggest that OT may play a role in the regulation of oviductal functions (Schaeffer *et al.* 1984), and stimulate oviduct motility involved with successful egg collection and/or sperm embryo transport, as postulated in the ewe (Wallace *et al.* 1991). However, we recently reported that the local distribution of OT in the bovine oviduct was low,

and did not vary with the stage of the estrous cycle (Wijayagunawardane *et al.* 1998). The novel inhibitory effect of OT on the contractile mechanisms in the oviduct observed in the present study strongly suggests a possible inhibitory action of luteal OT on the LH-induced oviductal contraction through the suppression of the LH-stimulated PGE₂, PGF_{2α} and ET-1 secretion during the postovulatory period. Although the possible mechanisms involved in this phenomenon are unclear, the inhibitory effect of OT may be due to interaction of LH and OT at the receptor, COX or PK level. Further study is needed to clarify these points.

In conclusion, the overall results suggest that the pre-ovulatory LH surge, together with increasing E₂ secretion

Figure 6 The relative amount of mRNA (integrated absorbance; means $\pm$ S.E.M.) is depicted for each PCR result: (A) ET-1 PCR, (B) ET-R A PCR and (C) ET-R B PCR ($n=4$). Different letters above the bars denote significance at $P<0.05$.

from the Graafian follicle and basal P_4 levels from the regressing CL, induce maximum stimulation of oviductal production of PG and ET-1, resulting in an active oviduct contraction. Moreover, ET-1 may be a local mediator of LH modulation of oviduct PG synthesis. OT released from

the newly formed CL appears to block these mechanisms, thus inhibiting the contraction to secure slow transport of the embryo to the uterus.

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References

- Acosta TJ, Miyamoto A, Ozawa T, Wijayagunawardane MPB & Sato K 1998 Local release of steroid hormones, prostaglandin E_2 and endothelin-1 from bovine mature follicles *in vitro*: effects of luteinizing hormone, endothelin-1 and cytokines. *Biology of Reproduction* **59** 437–443.
- Atanackovic D, Frkovic A, Mirosevic L & Frkovic A 1983 The effect of prostaglandin $F_{2\alpha}$ and indomethacin on the longitudinal musculature of the human oviduct. *Jugoslavian Ginekologiju i Opstetriciju* **23** 112–115.
- Ayad V, Guldenaar S & Wathes D 1991 Characterization and localization of oxytocin receptors in the uterus and oviduct of the non-pregnant ewe using an iodinated receptor antagonist. *Journal of Endocrinology* **128** 187–195.
- Barber D, Sieck G, Fitzpatrick L & Miller V 1996 Endothelin receptors are modulated in association with endogenous fluctuations in estrogen. *American Journal of Physiology* **271** H1999–2006.
- Barbosa I, Maia HJ, Dourado V & Coutinho E 1980 Effect of prostaglandin $F_{2\alpha}$ on oviduct contractility in marmoset monkeys. *Fertility and Sterility* **33** 197–200.
- Bennett W, Watts T, Blair W, Waldhalm S & Fuquay J 1988 Patterns of oviductal motility in the cow during the oestrous cycle. *Journal of Reproduction and Fertility* **83** 537–543.
- Chomczynski P & Sacchi N 1987 Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Analytical Biochemistry* **162** 156–159.
- Collett G, Kohnen G, Campbell S, Davenport A, Jeffers M & Cameron I 1996 Localization of endothelin receptors in human uterus throughout the menstrual cycle. *Molecular Human Reproduction* **2** 439–444.
- Derecka K, Pietila E, Rajaniemi H & Ziecik A 1995 Cycle dependent LH/hCG receptor gene expression in porcine nongonadal reproductive tissues. *Journal of Physiology and Pharmacology* **46** 77–85.
- Emori T, Hirata Y, Ohta K, Kanno K, Eguchi S, Imai T, Shichiri M & Marumo F 1991 Cellular mechanism of endothelin-1 release by angiotensin and vasopressin. *Hypertension* **18** 165–170.
- Gabler C, Lauer B, Einspanier A, Schams D & Einspanier R 1997 Detection of mRNA and immunoreactive proteins for acidic and basic fibroblast growth factor and expression of the fibroblast growth factor receptors in the bovine oviduct. *Journal of Reproduction and Fertility* **109** 213–221.
- Gabler C, Plath-Gabler A, Einspanier A & Einspanier R 1998 Insulin-like and fibroblast growth factors and their receptors are differentially expressed in the oviducts of the common marmoset monkey (*Callithrix jacchus*) during the ovulatory cycle. *Biology of Reproduction* **58** 1451–1457.

- Gilbert C, Cripps P & Wathes D 1992 Effect of oxytocin on the pattern of electromyographic activity in the oviduct and uterus of the ewe around oestrus. *Reproduction, Fertility and Development* **4** 193–203.
- Halbert S & Conrad J 1975 *In vitro* contractile activity of the mesotubarium superius from the rabbit oviduct in various endocrine states. *Fertility and Sterility* **26** 248–256.
- Han S, Lei Z & Rao C 1996 Up-regulation of cyclooxygenase-2 gene expression by chorionic gonadotropin in mucosal cells from human fallopian tubes. *Endocrinology* **137** 2929–2937.
- Ireland J, Murphee R & Coulson P 1980 Accuracy of predicting stages of bovine estrous cycle by gross appearance of the corpus luteum. *Journal of Dairy Science* **63** 155–160.
- Kisielewska J, Flint A & Ziecik A 1996 Phospholipase C and adenylate cyclase signalling systems in the action of hCG on porcine myometrial smooth muscle cells. *Journal of Endocrinology* **148** 175–180.
- Labadia A, Costa G, Jimenez E, Triguero D & Garcia-Pascual A 1997 Endothelin receptor-mediated Ca^{2+} mobilization and contraction in bovine oviductal arteries: comparison with noradrenaline and potassium. *General Pharmacology* **29** 611–619.
- Lei Z, Toth P, Rao C & Pridham D 1993 Novel coexpression of human chorionic gonadotropin (hCG)/human luteinizing hormone receptors and their ligand hCG in human fallopian tubes. *Journal of Clinical Endocrinology and Metabolism* **77** 863–872.
- Lindblom B & Andersson A 1985 Influence of cyclooxygenase inhibitors and arachidonic acid on contractile activity of the human Fallopian tube. *Biology of Reproduction* **32** 475–479.
- Lindblom B, Ljung B & Hamberger L 1979 Adrenergic and novel non-adrenergic neuronal mechanisms in the control of smooth muscle activity in the human oviduct. *Acta Physiologica Scandinavica* **106** 215–220.
- Lundin S, Forman A, Rechberger T, Svane D & Andersson K 1989 Immunoreactive oxytocin and vasopressin in the non-pregnant human uterus and oviductal isthmus. *Acta Endocrinologica* **120** 239–244.
- Miyamoto A & Schams D 1991 Oxytocin stimulates progesterone release from microdialyzed bovine corpus luteum *in vitro*. *Biology of Reproduction* **44** 1163–1170.
- Miyamoto A, Tashiro Y, Nakatsuka T, Meyer H, Taguchi K, Abe N & Fukui Y 1995 Effect of tumor necrosis factor- α and interleukin-1 on local release of progesterone, prostaglandin $\text{F}_{2\alpha}$ and oxytocin in microdialyzed ovine corpus luteum *in vitro*. *Assisted Reproductive Technology/Andrology* **8** 21–32.
- Miyamoto A, Kobayashi S, Arata S, Ohtani M, Fukui Y & Schams D 1997 Prostaglandin $\text{F}_{2\alpha}$ promotes the inhibitory action of endothelin-1 on the bovine luteal function *in vitro*. *Journal of Endocrinology* **152** R7–R11.
- Orlicky D & Williams-Skipp C 1993 Immunohistochemical localization of $\text{PGF}_{2\alpha}$ receptor in the rat oviduct. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **48** 185–192.
- Rao C, Li X, Toth P, Lei Z & Cook V 1993 Novel expression of functional human chorionic gonadotropin/luteinizing hormone receptor gene in human umbilical cords. *Journal of Clinical Endocrinology and Metabolism* **77** 1706–1714.
- Rodriguez-Martinez H, Einarsson S & Larsson B 1982a Spontaneous motility of the oviduct in the anaesthetized pig. *Journal of Reproduction and Fertility* **66** 615–624.
- Rodriguez-Martinez H, Einarsson S, Larsson B, Akusu M & Settergren I 1982b Spontaneous motility of the pig oviduct *in vitro*. *Biology of Reproduction* **26** 98–104.
- Rosselli M, Imthurn B, Macas E & Keller P 1994 Endothelin production by bovine oviduct epithelial cells. *Journal of Reproduction and Fertility* **101** 27–30.
- Ruckebusch Y & Bayard F 1975 Motility of the oviduct and uterus of the cow during the oestrous cycle. *Journal of Reproduction and Fertility* **43** 23–32.
- Schaeffer J, Liu J, Hsueh A & Yen S 1984 Presence of oxytocin and arginine vasopressin in human ovary, oviduct, and follicular fluid. *Journal of Clinical Endocrinology and Metabolism* **59** 970–973.
- Schramm W, Einer-Jensen N, Schramm G & McCracken J 1986 Local exchange of oxytocin from the ovarian vein to ovarian arteries in sheep. *Biology of Reproduction* **34** 671–680.
- Shemesh M, Gurevich M, Mizrahi D, Dombrowski L, Stram Y, Fields M & Shore L 1997 Expression of functional luteinizing hormone (LH) receptor and its messenger ribonucleic acid in bovine uterine veins: LH induction of cyclooxygenase and augmentation of prostaglandin production in bovine uterine veins. *Endocrinology* **138** 4844–4851.
- Spilman C & Harper M 1975 Effects of prostaglandins on oviductal motility and egg transport. *Gynecological Investigation* **6** 186–205.
- Sun T, Lei Z & Rao C 1997 A novel regulation of the oviductal glycoprotein gene expression by luteinizing hormone in bovine tubal epithelial cells. *Molecular and Cellular Endocrinology* **131** 97–108.
- Toth P, Li X, Lei Z & Rao C 1996 Expression of human chorionic gonadotropin (hCG)/luteinizing hormone receptors and regulation of the cyclooxygenase-1 gene by exogenous hCG in human fetal membranes. *Journal of Clinical Endocrinology and Metabolism* **81** 1283–1288.
- Tsunoda H, Miyauchi T, Fujita K, Kubo T & Goto K 1993 Mechanism of rat uterine smooth muscle contraction induced by endothelin-1. *British Journal of Pharmacology* **110** 1437–1446.
- Wallace J, Helliwell R & Morgan P 1991 Autoradiographical localization of oxytocin binding sites on ovine oviduct and uterus throughout the oestrous cycle. *Reproduction, Fertility and Development* **3** 127–135.
- Weber J, Freeman D, Vanderwall D & Woods G 1991 Prostaglandin E_2 hastens oviductal transport of equine embryos. *Biology of Reproduction* **45** 544–546.
- Wijayagunawardane MPB, Cerbito WA, Miyamoto A, Acosta TJ, Takagi M, Miyazawa K & Sato K 1996 Oviductal progesterone concentration and its spatial distribution in cyclic and early pregnant cows. *Theriogenology* **46** 1149–1158.
- Wijayagunawardane MPB, Miyamoto A, Cerbito WA, Acosta TJ, Takagi M & Sato K 1998 Local distributions of oviductal estradiol, progesterone, prostaglandins, oxytocin and endothelin-1 in the cyclic cow. *Theriogenology* **49** 607–618.

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