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Cyclooxygenase-2 and prostaglandin E(2)(PGE(2)) receptor messenger RNAs are affected by bovine oocyte maturation time and cumulus-oocyte complex quality, and PGE(2) induces moderate expansion of the bovine cumulus in vitro.

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Cyclooxygenase-2 and Prostaglandin E_2(PGE_2) Receptor Messenger RNAs Are Affected by Bovine Oocyte Maturation Time and Cumulus-Oocyte Complex Quality, and PGE_2 Induces Moderate Expansion of the Bovine Cumulus In Vitro[1]

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ABSTRACT

Expression of cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2) receptor 2 (EP2) are necessary for rodent cumulus expansion in vivo. Prostaglandin E_2 receptor 3 (EP3) has been detected in bovine preovulatory follicles and corpora lutea. The current experiments examined the effect of PGE_2 on bovine cumulus expansion in vitro and expression of COX-2, EP1, EP2, EP3, and EP4 mRNAs in bovine cumulus-oocyte complexes (COCs) at 0, 6, 12, 18, and 24 h time points during maturation in vitro. Concentrations of PGE_2 above 50 ng/ml resulted in moderate cumulus expansion of bovine COCs, but expansion did not occur in the absence of serum. COX-2 mRNA expression increased in bovine COCs at 6 h and 12 h of maturation, then decreased. EP2 mRNA was detectable by reverse transcription-polymerase chain reaction at all time points. EP3 mRNA expression increased in COCs from 0 to 6 h and remained at this higher level through the culture period. Very low levels of EP4 mRNA expression were detectable, but EP1 was not detected in bovine COCs. Because EP receptor mRNAs and COX-2 mRNA are expressed in bovine COCs, there exists the potential for a prostaglandin autocrine/paracrine regulatory pathway during oocyte maturation. Differential expression of the EP3 mRNA among varying COC classes indicates that this gene product may be a useful marker of oocyte competence. Although the PGE_2 pathway is involved in cumulus expansion, serum factors are required to mediate PGE_2-induced expansion.

cumulus cells, gene regulation, in vitro fertilization, oocyte development, ovary

INTRODUCTION

Early experiments have shown a role for prostaglandin E_2 (PGE_2) in cumulus expansion in vitro in mice [1] and rats [2]. There are two isoforms of an enzyme known to control the prostaglandin synthesis pathway: the constitutively active cyclooxygenase-1 (COX-1) and the inducible cyclooxygenase-2 (COX-2) [3, 4]. Although both COX-1 and COX-2 mRNAs are expressed in the uterus during the time of implantation [5], COX-1 knockouts display normal fertility but have difficulties in parturition [6], while COX-2 knockouts have defects in ovulation, fertilization, decidualization, and implantation [7]. In rodents, COX-2 protein is first detected in the granulosa of eCG-induced preovulatory follicles following hCG treatment [8, 9]. In cattle, COX-2 protein is expressed in the granulosa of some preovulatory follicles by 18 h after hCG, and more preovulatory follicles express COX-2 protein by 24 h [10]. COX-1 protein is not induced by hCG in bovine preovulatory follicles [11]. Thus, the COX-2 isoform is more likely than the COX-1 isoform to have an important role in early reproductive events in cattle, including oocyte maturation, ovulation, and fertilization.

Prostaglandin E_2 concentrations are very low in bovine follicular fluid before the gonadotropin surge and remain low at 18 h but increase to 13 ng/ml in preovulatory follicles 24 h after hCG [10]. Systemic treatment with PGE_2 restores the ability of COX-2 knockout mice to ovulate [9]. A small amount of PGE_2 is synthesized by maturing bovine cumulus-oocyte complexes (COCs) in vitro [12, 13]. However, the predominant source of intrafollicular PGE_2 is granulosa cells [14]. Although murine granulosa cells secrete sufficient PGE_2 to induce cumulus expansion, cumuli will not expand in the absence of serum [14]. Serum factors are required for hyaluronic acid retention within the expanding cumulus [1, 15, 16]. In recent years, this serum factor was determined to be of the inter-α-inhibitor family (IαI) [16, 17].

Prostaglandin E_2 receptors are members of the G-type receptor family; there are currently four subtypes (EP1–EP4) known that activate different and often conflicting pathways. Activation of EP1 and EP3 generally results in contraction of smooth muscle, while activation of EP2 and EP4 receptors generally results in relaxation; activation of EP2 and EP4 receptors increase cAMP, EP1 increases Ca^{2+} mobilization, EP3A decreases cAMP, EP3B and C isoforms increase cAMP, and EP3D increases Ca^{2+} [18]. EP2 has recently been detected in the mouse cumulus [19], targeted deletions for EP2 have been generated by three groups, and all null mouse lines have defects in ovulation and fertilization [19–21] with one strain displaying defects in cumulus expansion [19]. In addition, another PGE receptor mRNA (EP3) has been recently detected in bovine and ovine preovulatory follicles and corpora lutea [22, 23].

For over 20 yr, research has focused on the induction of bovine oocyte maturation in vitro. However, with even the best systems developed thus far, including maturation in TCM-199 (tissue culture media) containing serum, gonadotropins, and estradiol at 38.5°C in 5% CO_2 in air, and embryo culture in TCM-199 or cSOFMaa (citrate supple-

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TABLE 1. Primers for COX-2 and bovine prostaglandin E_2 receptors.

Product	5′	3′	Temperature (°C)	Size (bp)
COX-2	GCCTGGTCTGATGATGTATGC	GGTGAGGTGCGTATCTTGAAC	54	880
EP1	CCAGCTCGTAGGCATCATGG	CTTGAAGGCAGCAGGCGAAC	57	216
EP2	CCTGCAACTTCAGCGTCAT	TCTAAGAGCTTGGAGGTCCC	54	251
EP3	CAAGCCACATGAAGACCAGC	TCATTATCAGCAACGGCGAC	58	408
EP4	CAAGGAGCAGAAGGAGACGA	GGCTGTAGAAGTAGGCGTGG	57	248

mented synthetic oviductal fluid media with amino acids) in a reduced O_2 gas atmosphere; only 30%–35% of immature COCs collected develop to the blastocyst stage [24, 25]. Therefore, the purpose of these experiments was to investigate the role of PGE_2 and serum factors in inducing bovine cumulus expansion in vitro. In addition, expression of COX-2, EP1, EP2, EP3, and EP4 mRNAs was examined in bovine COCs of differing qualities during maturation in vitro.

MATERIALS AND METHODS

Bovine COCs were isolated by follicular aspiration using an 18-gauge needle connected to a vacuum system from slaughterhouse ovaries provided by ABEL Laboratories (Guelph, ON, Canada). Serum-free TCM-199 (Gibco BRL, Burlington, ON, Canada), buffered with Hepes and bicarbonate, with 50 IU/ml heparin (Leo Pharma, Ajax, ON, Canada) was used for washing COCs. Pools of 35–65 COCs were matured in vitro in 0.5 ml TCM-199 containing bicarbonate and pyruvate in four-well plates (Nalge Nunc International, Roskilde, Denmark) at 38.5°C in a 5% CO_2 in air atmosphere. There were seven replicates with a total of about 400 COCs/treatment. Serum (newborn calf serum) was obtained from Gibco BRL. PGE_2 (Cayman Chemical, Ann Arbor, MI) was dissolved in 100% ethanol to produce a stock solution of 100 ng/μl. COCs were evaluated for cumulus expansion at 21–22 h of maturation as I, little to no expansion; II, moderate expansion; and III, full expansion [26].

For analysis of COX-2, EP1, EP2, EP3, and EP4 mRNAs, COCs were matured in TCM-199 containing pyruvate, pFSH (1 μg/ml FSH; Vetrepharm, Ottawa, ON, Canada), oLH (5 μg/ml LH; Vetrepharm), estradiol-17β (1 μg/ml estradiol-17β; Sigma-Aldrich, Oakville, ON, Canada), and 10% serum. COCs were divided into three quality grades prior to placement in maturation media, similar to previous reports [27, 28]. Quality grade one consisted of COCs with even oocyte granulation and multiple layers of cumulus cells, quality grade two COCs had uneven granulation of the oocyte or only one or two layers of cumulus cells, while quality grade three COCs had several defects: small oocyte, very uneven granulation of oocyte, cumulus already expanded or incomplete or absent cumulus cell layers. In previous experiments, small oocytes have been reported to be incompetent to complete meiosis [29], and in other experiments, developmental rates have been reported to be lower with poorer quality oocytes [28], thus this group may be expected to have altered mRNA expression levels. The experiment was replicated four times. Aliquots of COCs (50) of each quality grade were removed and placed into 0.5-ml microcentrifuge tubes at 0 h (following final wash) or 6, 12, 18, or 24 h after dishes were placed into the incubator for maturation. Excess medium was removed after brief centrifugation, and COCs were frozen in liquid nitrogen before being transferred to a −70°C freezer. Total RNA was extracted from COCs with 24 μg *Escherichia coli* rRNA (Roche Molecular Biochemicals, Laval, PQ, Canada) as a carrier, by using a standard phenol/chloroform extraction method [30]. The RNA samples were quantified by measuring 260/280 nm OD absorbance using spectrophotometry. For analysis of COX-2, EP2, EP3, and EP4 expression, RNA samples representing 40 COC equivalents were reverse transcribed as reported previously [31] with oligo $(dT)_{12–18}$ primer (Gibco BRL) and Superscript (Gibco BRL), except the volume was doubled to 40 μl (1 COC/μl). Just prior to reverse transcription, 0.1 pg rabbit globin mRNA (Gibco BRL) was added per COC equivalent [31]. For EP1, RNA samples were reverse transcribed using random hexamers (2 μl/20 μl reaction; Boehringer-Mannheim, Germany). RNA from positive control bovine tissues (liver for β-actin, kidney for COX-2 and EP2, lung for EP4, corpus luteum for EP3) was extracted using a standard phenol/chloroform technique, and 1–2.5 μg of RNA was reverse transcribed as above. For EP1, kidney was reverse transcribed with random hexamers.

The β-actin primers were as reported previously [31]. These primers amplify a 243-base pair (bp) product at an annealing temperature of 56°C for 37 cycles and, because they are intron-spanning, were used to detect possible genomic contamination (408-bp product). The α-globin primers were as reported previously [31] and amplified a product of 257 bp at 55°C for 30 cycles. Primers for COX-2 and EP1–EP4 are in Table 1. Bovine primers for COX-2 were designed from the published sequence (Genbank accession number AF031698). The primers amplified a product of 880 bp at 54°C. EP1 primers were designed from a partial ovine sequence (Genbank accession number AF035415) [32], and these primers amplified a product of the expected size of 216 bp from kidney at 57°C for 45 cycles; however, a product was never amplified from COCs despite variations made in $MgCl_2$, cycle number, and temperature. The EP2 primers were designed to amplify a conserved region of EP2 mRNA by running a sequence comparison of rat, mouse, human, ovine, and canine sequences (Genbank accession numbers U48858, D50589, U19487, AF035416, and AF075602). Primers amplified a product of 251 bp at 54°C. Sequences for bovine EP3 [33] (Genbank accession numbers D21345, D21346, D21347, D21348) were known and primers were designed from these sequences. The EP3 primers amplified a single product of 408 bp (common to all isoforms) at 58°C. EP4 primers were designed from a partial ovine sequence (Genbank accession number AF035418) [32]. The EP4 primers amplified a product of 248 bp at 57°C for 40 cycles. All primers were synthesized by Gibco BRL. The identity of the COX-2, EP2, EP3, and EP4 products was confirmed by dye-deoxysequencing (Robarts Research Institute, London, ON, Canada).

COX-2, EP2, and EP3 mRNAs were subjected to reverse transcription-polymerase chain reaction (RT-PCR) and agarose gel electrophoresis. Bands were quantified by comparing the ratio of band intensity to the α-globin standard [31, 34]. EP4 mRNAs were not quantified due to low abundance. Two microliters of cDNA (two COC equivalents) was added to each PCR reaction; for positive control tissues, 1–2 μl cDNA was added per tube. PCRs were carried out with 10× PE Gold buffer (Perkin-Elmer, Canada Ltd., Mississauga, ON, Canada) with a final concentration of 1.25–2 mM $MgCl_2$ (Perkin Elmer), primer concentration 1 μM (2 μM for actin), 200 μM dNTPs (Gibco-BRL), and 1 unit Ampli*Taq* Gold DNA Polymerase (Perkin-Elmer). The basic program included a soak of 95°C for 10 min, followed by a cycle program of 95°C for 1 min, gene-specific annealing temperature for 30 sec, and 72°C for 1 min, followed by a final extension at 72°C for 10 min. The numbers of cycles required to achieve amplification in the linear range were tested in a preliminary experiment. All PCR reactions were done in Perkin-Elmer GeneAmp 2400 thermocyclers (PE Applied Biosystems, Mississauga, ON, Canada). Twenty microliters of product was resolved on a 2% agarose gel containing ethidium bromide and a Gene-Ruler 100-bp DNA ladder (MBI Fermentas Inc., Flamborough, ON, Canada). Photographs were taken with a UV camera system (Amersham Pharmacia Biotech, Baie d'Urfe, PQ, Canada). Image analysis and quantification was done with the ImageMaster VDS program (Amersham Pharmacia Biotech).

Statistical Analyses

For studying the effects of PGE_2 on expansion, χ^2 tests were performed according to the method of Snedecor [35]. For gene expression, multiple ANOVA was performed using the Practical Statistics program (Canadian Academic Technology Inc., West Flamborough, ON, Canada) with main effects of quality grade and time of maturation. There was no interaction between grade and time effects. Where significant main effects existed, mean separation procedures were performed to detect differences.

RESULTS

Effect of PGE_2 on Bovine Cumulus Expansion

An experiment was carried out to determine whether, in agreement with rodent data, PGE_2 has an effect on expan-

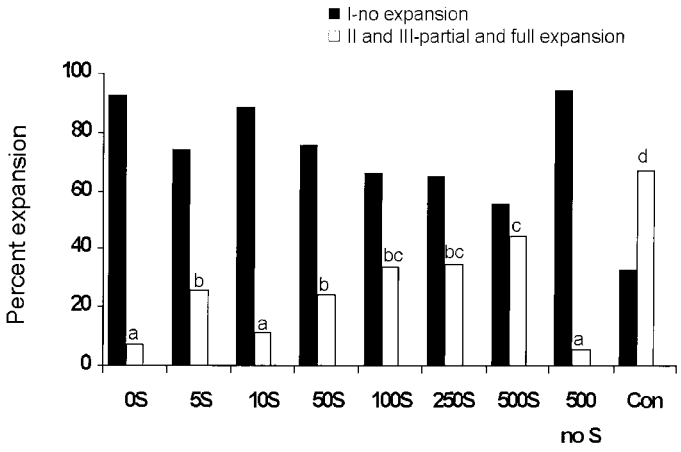

FIG. 1. Percent cumulus expansion of bovine COCs matured 24 h in vitro with varying doses of PGE_2 (ng/ml) with and without 10% serum compared to positive control COCs matured in TCM-199 containing 10% serum and 1 μg/ml FSH, 5 μg/ml LH, and 1 μg/ml estradiol-17β. I is no expansion, II is moderate expansion, III is full expansion. χ^2 tests were performed to determine differences in the percentage of COCs that underwent partial or full expansion (II or III). [a–d]Values with no superscripts in common are different at $P < 0.05$.

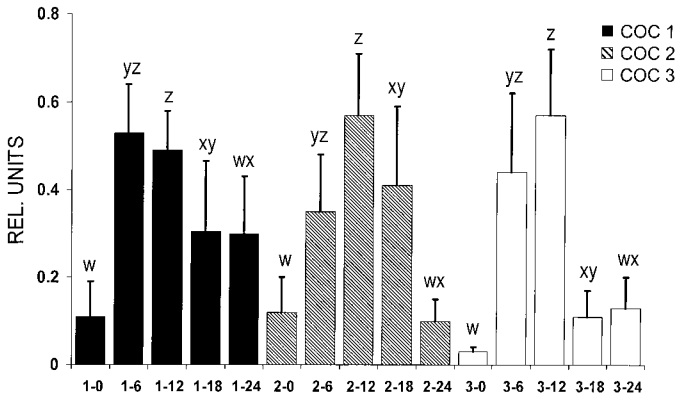

FIG. 3. Expression of COX-2 mRNA in three quality grades of bovine COCs during maturation in vitro. Solid bars: Expression in quality grade 1 COCs at 0, 6, 12, 18, and 24 h, respectively. Hatched bars: Expression in quality grade 2 COCs at 0, 6, 12, 18, and 24 h, respectively. White bars: Expression in quality grade 3 COCs at 0, 6, 12, 18, and 24 h, respectively. Mean ± SEM (n = 4 replicates). [w–z]Time points with no superscripts in common are different at $P < 0.05$.

sion of bovine COCs in vitro. A dose-dependent effect of PGE_2 was seen on bovine cumulus expansion in the presence of 10% serum. Doses of PGE_2 above 50 ng/ml consistently increased expansion of bovine COCs (Fig. 1). However, COCs matured with the highest PGE_2 dose tested, 500 ng/ml, did not achieve expansion equivalent to that of COCs matured in media containing serum and gonadotropins. Bovine COCs did not expand when cultured with 500 ng/ml PGE_2 in the absence of serum.

Effect of COC Quality and Maturation on Expression of COX-2, EP2, and EP3 mRNAs

Because PGE_2 was shown to induce cumulus expansion, it was necessary to determine whether COX-2 mRNA, an enzyme required for synthesis of PGE_2, was present in the cumulus cells, which could indicate an autocrine action. In addition, it was important to determine which PGE_2 receptor mRNAs were present and could be involved in signaling in the bovine COC and the time course of expression during in vitro maturation. Representative pictures of PCR-amplified products are shown in Figure 2. Weak expression of COX-2 mRNA was detected by RT-PCR in bovine COCs of all COC quality grades at 0 h (Fig. 3), prior to initiation of maturation. There was no effect of COC quality grade on expression of COX-2 mRNA. However, there was an overall effect of maturation time ($P < 0.002$), with increased expression at 6–12 h of maturation compared to 0 h ($P < 0.002$, Fig. 3). Expression decreased and was again lower at 24 h than at 6 and 12 h ($P < 0.02$). Increased COX-2 mRNA expression was apparent in COCs at 6 h, which was prior to morphological evidence of cumulus expansion (12 h).

The partial sequence of bovine EP2 is reported in Figure 4. EP2 mRNA was detected by RT-PCR in bovine COCs of all quality grades at 0 h of oocyte maturation (Fig. 5). There were no differences among COC quality grades, but

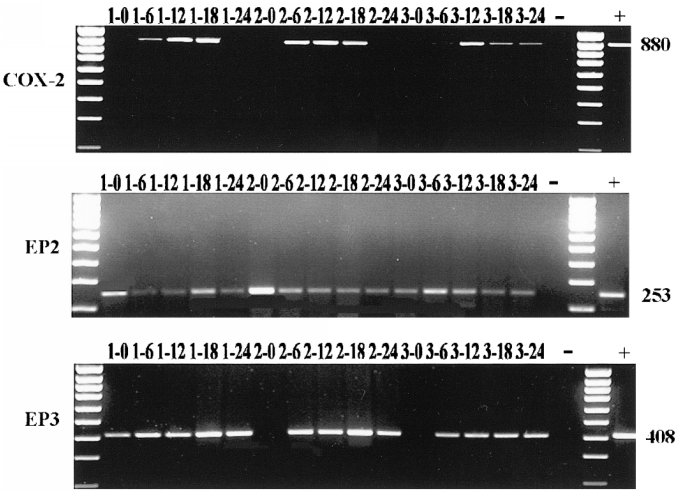

FIG. 2. Representative RT-PCR figures with gene on the left and PCR product size in bp on right. The leftmost lane is the 100-bp DNA marker lane followed by oocyte quality 1, hour 0; quality 1, hour 6, etc. The (−) lane is the PCR blank that contains all components except cDNA. This is followed by another marker lane and then a (+) positive tissue control lane.

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23  EP2

24  cctgcaacttcagcgtcatcgtcaacctcatccacatgcaccgccggggcaggagaagcc 60
25  ||||||||||||||||||||||||||||||||| |||||||||||||||||||||||||
26  cctgcaacttcagcgtcatcgtcaacctcatccgcatgcaccgccggggcaggagaagcc 67
27
28
29  gctgcggaccctccttgggca-gca-gccacaggagagcggaaagggtatccatggcgga 118
30  ||||||||||||||||||||| ||| ||||| ||||||||||||||||| ||||||||||
31  gctgcggaccctccttgggcaagcatgccacgggagagcggaaagggcatccatggcgga 127
32
33
34  ggagacggaccatcttattctcctggctatcatgaccatcaccttcgccgtctgctcctt 178
35  ||||||||||||||||||| ||||| ||||||||||||||||||||||||||||||||||
36  ggagacggaccatcttattcttctggccatcatgaccatcaccttcgccgtctgctcctt 187
37
38
39  gcctttcacgatttttgcatatatgaatgaaaactcttcccggaaagaaaagtgggacct 238
40  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
41  gcctttcacgatttttgcatatatgaatgaaaactcttcccggaaagaaaagtgggacct 247
42
43
44  ccaagctcttaga 251
45  |||||||||||||
46  ccaagctcttaga 260
47
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FIG. 4. Partial sequence of bovine EP2. The PCR-amplified product has 97% homology at the nucleotide level to the partial ovine EP2 sequence (AF035416). Bovine sequence is on top, ovine on the bottom. Dashes indicate identical bases; gaps indicate nucleotide differences from the ovine sequence.

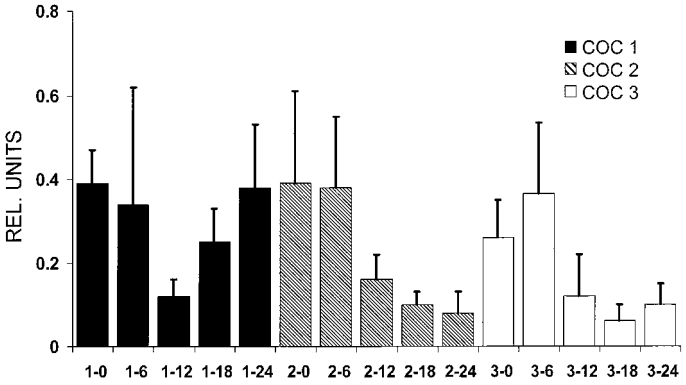

FIG. 5. Expression of EP2 mRNA in three quality grades of bovine COCs during maturation in vitro. Solid bars: Expression in quality grade 1 COCs at 0, 6, 12, 18, and 24 h, respectively. Hatched bars: Expression in quality grade 2 COCs at 0, 6, 12, 18, and 24 h, respectively. White bars: Expression in quality grade 3 COCs at 0, 6, 12, 18, and 24 h, respectively. Mean ± SEM (n = 4 replicates).

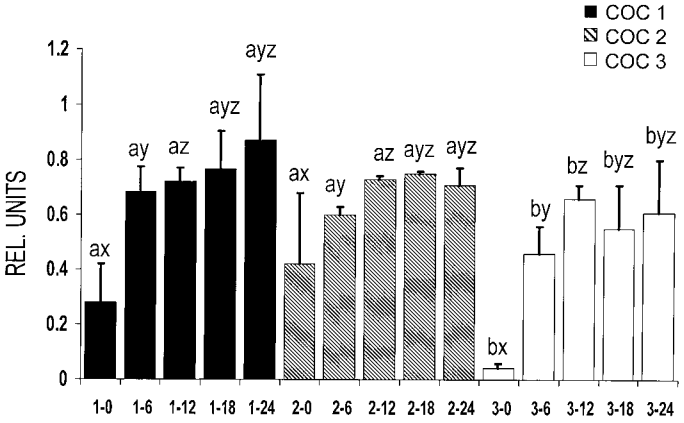

FIG. 6. Expression of EP3 mRNA in three quality grades of bovine COCs during maturation in vitro. Solid bars: Expression in quality grade 1 COCs at 0, 6, 12, 18, and 24 h, respectively. Hatched bars: Expression in quality grade 2 COCs at 0, 6, 12, 18, and 24 h, respectively. White bars: Expression in quality grade 3 COCs at 0, 6, 12, 18, and 24 h, respectively. Mean ± SEM (n = 4 replicates). ^{ab}COC quality grades with no superscripts in common are different, at least $P < 0.05$. $^{x-z}$Time points with no superscripts in common are different, at least $P < 0.05$.

there was an overall trend ($P < 0.07$) toward decreased expression throughout the maturation period.

There was an overall effect of COC quality grade on abundance of EP3 mRNA (Fig. 6). Higher expression of EP3 mRNA was detected in COC quality grades 1 and 2 than in quality grade 3 COCs ($P < 0.003$ and $P < 0.05$, respectively). There was an overall effect of maturation time on abundance of EP3 mRNA ($P < 0.003$). Expression of EP3 mRNA was lowest at 0 h but increased and remained elevated from 6 to 24 h of maturation (all at least $P < 0.02$). The partial sequence of bovine EP4 is reported in Figure 7. EP4 was detected inconsistently at low levels in bovine COCs (data not shown).

DISCUSSION

Doses of 100 ng/ml PGE_2 caused maximal expansion of mouse COCs in vitro in the presence of serum [1]. It is reported that preovulatory bovine follicular fluid concentrations of PGE_2 increase to 13 ng/ml after hCG [10] or 88 ng/ml 20 h after an endogenous LH surge [36]. A significant increase in cumulus expansion was observed in bovine COCs matured in vitro with concentrations of PGE_2 over 50 ng/ml but only in the presence of serum. In the absence of serum, there was little cumulus expansion of bovine COCs with 500 ng/ml PGE_2. Rat COCs failed to expand with 352 ng/ml PGE_2 in the presence of fetal bovine serum [37], but the same PGE_2 concentration induced cumulus expansion in the presence of granulosa cells [2]. It is possible that incompatibility between serum and factors produced by the expanding cumuli of different species could affect response to PGE_2, as it has been reported that the composition of serum IαIs differ among species [38, 39]. It is not known what the granulosa contributes to cumulus expansion in vivo; however, the bovine COCs employed in these experiments were not cocultured with granulosa cells during in vitro maturation.

In rats, COX-2 protein was induced in preovulatory follicles only by hCG, not by FSH or E_2 treatments [8]. COX-2 protein and mRNA expression increased in preovulatory bovine granulosa cells in vivo by 16–24 h after hCG or the endogenous LH surge [10, 11, 22, 36]. COX-2 immunoreactivity increased 79-fold in granulosa cells at 24 h compared to 0 h of estrus [36], while the mRNA was reported to increase 3000-fold in the same time period [22]. In addition, COX-2 mRNA expression increased in bovine granulosa cells cultured with serum or hCG [22]. It has not often been reported whether COX-2 is also expressed in cumulus cells; however, one study reported intense COX-2 immunoreactivity in mouse cumulus as well as in mural granulosa 8 h after hCG [9]. This is the first instance in which COX-2 mRNA was reported to be expressed in bovine COCs. In the current experiment, COX-2 mRNA was detected by RT-PCR in bovine COCs at 0 h, prior to placement in maturation culture. Expression increased in all quality grades at 6–12 h of maturation, then decreased from 18 to 24 h (Fig. 3). In a previous study, the cumulus was reported to exhibit expansion in vivo prior to detection of COX-2 protein in the bovine follicle [10]. Because COX-2 mRNA was detected in bovine cumulus prior to expansion (this study) but is not detected in granulosa until after cumulus expansion [10], it is possible that COX-2 has two temporally distinct functions in bovine preovulatory follicles, the first being production of PGE_2 in the cumulus for expansion, and the second, to induce follicular rupture.

Recently, three groups have produced targeted gene deletions of the mouse PGE receptor 2 (EP2). Two strains

FIG. 7. A partial sequence of bovine EP4. The PCR-amplified product has 94% homology at the nucleotide level to the partial ovine EP4 sequence (AF035418). The bovine sequence is on top, ovine on the bottom. Dashes indicate identical bases, gaps indicate nucleotide differences from the ovine sequence.

have decreased ovulation rates [19, 21], and all three strains have severely impaired fertilization [19–21]. One mouse strain demonstrated defects in cumulus expansion [19], while another mouse strain was reported to be normal in this respect [20]. The severity of the EP2 phenotype may depend on the genetic background of the mice, as two strains also differed in blood pressure [20, 21]. It is thought that other EPs may potentially compensate for the lack of EP2 function in null mice [19, 20]. In the mouse strain with the most severe cumulus defect, the COCs failed to expand in vitro when cultured with PGE_2, although expansion was normal in response to gonadotropins [19]. Of the PGE_2 receptors, it was suspected that EP2 would have the most important role in the bovine ovary. The present experiments are the first to report expression of the EP2 receptor mRNA in bovine COCs. EP2 mRNA was detectable by RT-PCR from 0 to 24 h of maturation in bovine COCs, and there were no differences in expression among oocyte qualities. Although in mouse cumulus, EP2 mRNA expression increased following hCG [19], in bovine COCs there was a tendency toward decreased EP2 mRNA expression over maturation time.

In a recent paper, EP3 mRNA was detected at very low copy number in granulosa of preovulatory bovine follicles collected prior to the LH surge [22] and did not increase in granulosa cells of preovulatory follicles 24 h after an induced LH surge. However, expression of EP3 mRNA increased following in vitro culture of granulosa cells in media containing serum for 24 h [22]. This is the first time that EP3 mRNA has been reported to be present in bovine COCs. The EP3 sequence is not reported here, as it was 100% identical to the bovine EP3 sequence reported in Genbank. In the current experiments, expression of EP3 mRNA was detected in bovine COCs at 0 h. EP3 mRNA expression increased at 6 h, then remained at a fairly constant level throughout the culture period. EP3 mRNA was more highly expressed in COCs of the better quality grades; however, it is unclear whether increased EP3 expression is related to increased developmental competence of these oocytes. Perhaps the association of increased expression with increased COC quality was more evident with EP3 than EP2 and COX-2 mRNAs due to the lower variability of EP3 among replicates. The function of EP3 in COCs and corpora lutea is not clear, as mouse knockouts of EP3 do not display any obvious reproductive impairments [40], and expression of EP3 mRNA is higher in adrenal, myometrium, and heart tissue than in corpora lutea [23]. Possibly, increased expression of EP3 in COCs or granulosa is related to luteinization. Although EP4 mRNA is expressed at very low levels in bovine COCs, it is unlikely to be essential for reproduction, as surviving mouse EP4 knockouts were able to reproduce [41, 42]. Thus far, we have been unable to detect EP1 in bovine COCs but EP1 seems to be the PGE_2 receptor most restricted in expression and has only been detected in mouse kidney, lung, and hypothalamus [43, 44].

Several mRNAs such as COX-2 [10], LHr [45], and 3β-hydroxysteroid dehydrogenase [46] are not detectable in granulosa until bovine follicles reach at least 8–9 mm or potentially preovulatory in size. However, bovine COCs are commonly collected from follicles 2–8 mm in size for in vitro procedures. Oocytes from smaller follicles have a lower development rate than those from larger follicles [28, 47], and oocytes matured in vitro have lower developmental rates than ovulated oocytes [48, 49]. Little is known about gene expression in preovulatory COCs; therefore, it is not known whether current bovine in vitro maturation procedures induce equivalent mRNA expression (timing and amount) as in vivo-matured COCs. COCs collected from follicles maturing in vivo at various times from the LH surge would be the best controls to examine gene expression, but these would be difficult and expensive to collect. It is feasible to study how advancements in maturation conditions that improve development rate (gas atmosphere, hormones, growth factors, etc.) affect gene expression during maturation and culture in vitro.

Because COX-2 mRNA and protein are expressed in maturing COCs, and PGE_2 is detected in follicular fluid and culture media, it is likely the PGE_2 pathway is involved in oocyte maturation and cumulus expansion in vivo. As well, defects in cumulus expansion and fertilization in EP2 knockout mice suggest that this is an important pathway for female reproduction in vivo. However, it may not be as critical to achieve optimal cumulus expansion in vitro because the oocyte does not need to escape from the follicle. In addition, sperm numbers may be high enough to achieve satisfactory fertilization in vitro, whereas the lower sperm numbers in vivo require that the cumulus be sufficiently expanded to achieve optimal fertilization rates.

Despite many years of research, even the best maturation and oocyte culture systems result in the development in vitro of only 30–35% oocytes to the blastocyst stage [24, 25], which is substantially lower than the in vivo developmental rate. It is necessary to define marker genes that predict developmental competence of oocytes and embryos in vitro and in vivo and that would allow design of more appropriate maturation and culture media. This study is the first to examine expression of COX-2 and prostaglandin receptor mRNAs in bovine COCs and underscores that expression of these mRNAs is dependent on time of maturation and oocyte quality. The differential expression of the EP3 mRNA among varying COC qualities indicates that this gene product may be a useful marker to predict embryo development.

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