BIOLOGY OF REPRODUCTION **67,** 630–636 (2002)

Expression of Interleukin-1 (IL-1) System Genes in Equine Cumulus-Oocyte Complexes and Influence of IL-1β During In Vitro Maturation[1]

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ABSTRACT

A growing body of evidence suggests that the ovary is a site of inflammatory reactions, and thus, ovarian cells could represent sources and targets of the interleukin-1 (IL-1) system. The aim of the present work was to investigate the expression of IL-1α, IL-1β, IL-1ra, IL-1R1, and IL-1R2 genes in equine cumulus cells and oocytes. Moreover, the influence of IL-1β on in vitro maturation of cumulus-oocytes complexes (COCs) was examined. COCs were collected using ultrasound-guided follicular puncture in vivo. Oocytes and cumulus cells were isolated from preovulatory and subordinate follicles and were analyzed either at collection or after in vitro culture. An ultrasensitive reverse transcription-polymerase chain reaction was used to study the expression of IL-1 system members. In order to study the in vitro effect of IL-1β on oocyte nuclear maturation and cumulus expansion, immature COCs collected from subordinate follicles were cultured for 30 h in media containing or not containing IL-1β alone, or in combination with equine luteinizing hormone. Our results indicated that equine oocytes expressed IL-1β and IL-1R2 genes, and that cumulus cells expressed all IL-1 system members except IL-1α. In oocytes, IL-1β expression significantly decreased during in vitro culture compared with immature and mature oocytes analyzed at collection. Similarly, in cumulus cells, in vitro culture decreased IL-1ra and IL-1R1 expression. IL-1β was shown to significantly decrease the gonadotropin-induced nuclear maturation of oocytes. In conclusion, we demonstrated for the first time, in mares, the presence of IL-1β and IL-1R2 transcripts in oocytes and of IL-1β, IL-1ra, IL-1R1, and IL-1R2 in cumulus cells. The regulatory effect of IL-1β on COCs during maturation suggests that the IL-1 system could be of crucial importance for ovarian physiology in the mare.

cumulus cells, cytokines, ovary, ovum

INTRODUCTION

Interleukins are polypeptide cytokine components of the immune system that were originally defined by their action between leukocytes. Interleukin-1 (IL-1) was initially identified as a macrophage-derived lymphocyte-activating factor [1], and its inflammatory activity has been widely documented [2, 3]. IL-1 is organized as a gene system that includes two bioactive ligands, IL-1α and IL-1β [4]; two types of receptors, IL-1R1 and IL-1R2 [5, 6]; and a natural receptor antagonist, IL-1ra, which regulates IL-1 ligand biological activity [7]. Interleukins are produced by and are effective on a large number of tissues, including nonimmune cells, such as ovarian cells (for a review see [8]). In the ovary, IL-1 or IL-1-like bioactivity has been reported in human and porcine follicular fluid at the time of ovulation [9, 10]. Ex vivo, IL-1 has been shown to promote the ovulation process in rat [11] and rabbit [12] models. These observations led to the hypothesis that in mammalian species, IL-1 is a paracrine factor that could be involved in the cascade of events that lead to ovulation [13]. IL-1 is involved in several ovulation-associated events such as the synthesis of proteases [14], plasminogen activator [15, 16], and prostaglandins [17, 18] in the preovulatory follicle. IL-1α and IL-1β have been shown to stimulate gonadotropin-supported steroidogenesis in hamster, human, and rat granulosa cells [19–21]. By contrast, IL-1 may also negatively interfere with reproductive processes. Evidence of either the impairment of oocyte-spermatozoa interaction during in vitro fertilization or the inhibition of in vitro embryo development by IL-1β in mice has been shown [22]. However, IL-1β influences oocyte maturation, because it increases the rate of germinal vesicle breakdown in rabbit oocytes in an ovarian perfusion system [12].

In mares, the conditions for in vivo oocyte maturation differ somewhat from those observed in other domestic mammals and humans. The ovulatory surge of LH occurs rather as a progressive rise over several days, with a maximum at 1 day after ovulation [23, 24]. The requirements for in vitro oocyte maturation also seem to be unusual. The in vitro maturation rate in equine oocytes remains low in culture conditions adapted from those used in bovines [25], and in vitro fertilization gives only limited success [26]. Thus, oocyte maturation in mares could involve some species-specific mechanisms, including some related to the IL-1 system.

In this context, the purpose of the present work was to study the expression of IL-1 system gene products in equine oocytes and cumulus cells before and after in vitro and in vivo maturation using the semiquantitative, ultrasensitive reverse transcription-polymerase chain reaction (RT-PCR) methodology. Moreover, the role of the IL-1 system during cumulus-oocyte complex (COC) maturation was evaluated by studying the influence of IL-1β on in vitro oocyte meiotic resumption and cumulus expansion.

MATERIALS AND METHODS

Animals, Ovarian Monitoring, and Treatments

Adult cyclic pony mares in good body condition, kept indoors and fed with concentrates, were used for experiments from April to May. Mares received a prostaglandin $F_{2\alpha}$ analogue (Cloprostenol [Estrumate], 250 μg/mare i.m.; Pitman-Moore, Meaux, France) during the mid-luteal phase in order to induce luteolysis. Follicle growth was then assessed by routine rectal ultrasound scanning. An i.v. injection of 20 mg of crude equine gonadotropin (CEG) was performed when the largest follicle reached 33

[1]This work was supported by grants from Institut National de la Recherche Agronomique and Haras Nationaux. A.M. was supported by a fellowship from the Institut National de la Recherche Agronomique and the Région Centre of France.
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Received: 16 January 2002.
First decision: 8 February 2002.
Accepted: 12 March 2002.

ISSN: 0006-3363. http://www.biolreprod.org

TABLE 1. Primer sequences, hybridization temperature during PCR, and theoretical length of amplified fragments.

	Strand*	Primer (5′ to 3′)	Hybridization temperature (°C)	Length of amplified fragments (base pairs)
GAPDH	s	GTTTGTGATGGGCGTGAACC	62	255
	a-s	TTGGCAGCACCAGTAGGAGC		
β-Actin	s	AATCGTGCGTGACATCAAGG	60	212
	a-s	TAGTTTCGTGGATGCCACAG		
Interleukin-1α	s	GCCAATGATCCAGAAGAAGG	60	450
	a-s	CAGTTTTCCCTGCTTTGTGG		
Interleukin-1β	s	GGTGTTCTGCATGAGCTTTG	60	266
	a-s	TTTCTGCTTGAGAGGTGCTG		
Interleukin-1ra	s	TGCAAGATGCAAGCCTTCAG	60	425
	a-s	TGGAGGTAGAACTTGGTGAC		
Interleukin-1R1	s	TGATCTGCAATGTCACTGGC	60	372
	a-s	AATCCCTGTACCAAAGCACC		
Interleukin-1R2	s	ATGCTCTCTGGATTTTGCCG	62	311
	a-s	TACGAGTAAACGAGGGGGTC		

* s, Sense primer; a-s, antisense primer.

mm in diameter to induce ovulation [27]. This largest follicle is the preovulatory follicle, and is accompanied by subordinate follicles. Before follicle puncture, mares were sedated with detomidine (Domosedan; Pfizer, Amboise, France; 0.6 mg/100 kg body weight [BW] i.v.), and the rectum was relaxed with propantheline bromide (Sigma, Saint Quentin Fallavier, France; 60 mg/100 kg BW i.v.). After puncture sessions, mares were injected with an antibiotic (Intramicine; Rhône Mérieux, Lyon, France; 1 600 000 IU penicillin/100 kg BW and 1.3 g dihydrostreptomycin/100 kg BW i.m.).

Cumulus-Oocyte Complex Recovery

COCs were recovered from all follicles larger than 5 mm at the end of the follicular phase 34 h after CEG injection by transvaginal ultrasound-guided follicle puncture, as previously described [28]. Preovulatory and subordinate follicles were individually aspirated. Briefly, a single lumen needle was used to aspirate follicles larger than 25 mm in diameter, and a two-way needle was used to aspirate follicles smaller than 25 mm in diameter. After follicular fluid aspiration, each follicle was flushed several times with PBS (Dulbecco A; Unipath, Dardilly, France) containing 50 IU/ml of heparin (LEO A.A., Saint Quentin Yvelines, France) at 37°C, and scraped in order to pick up COCs. All aspirated fluids were examined with a stereomicroscope for COC recovery.

COCs were classified morphologically as either compact or expanded.

Oocyte Culture and Nuclear Examination

COCs from subordinate follicles were washed four times in PBS with 50 mg/L of gentamicin (Sigma). They were cultured individually in a humidified atmosphere (95% air and 5% CO_2) at 38.5°C for 30 h in 20 μl of maturation medium covered with mineral oil (Sigma). In the first experiment, three different maturation media were used. Medium 1 contained tissue culture medium 199 (TCM-199; Sigma) supplemented with 0.5% BSA (Sigma). Media 2 and 3 contained medium 1 supplemented, respectively, with either recombinant human interleukin-1β (rhIL-1β, 50 ng/ml; medium 2; Eurobio, Les Ulis, France) or mouse epidermal growth factor (EGF; Sigma; 50 ng/ml; medium 3). Medium 3 is routinely used in our laboratory for in vitro culture of equine oocytes [29, 30] and was considered as the positive control. In the second experiment, COCs were cultured individually in four different maturation media, media 1 and 2, or media 4 and 5. Media 4 and 5 contained medium 1 supplemented with either equine LH alone (eLH, 5 μg/ml) or in combination with rhIL-1β (50 ng/ml), respectively.

After culture, COCs were rinsed twice and stripped of their cumulus cells with a small glass pipette in PBS at 37°C. Entirely denuded oocytes were rinsed in PBS, stained with 1 μg/ml of bis-benzimide solution (Hoechst 33342; Sigma), and observed in a drop on a slide under a light and fluorescence microscope in order to determine the nuclear stage. Oocytes showing a polar body (light microscopy) and two distinct spots of chromosomes stained by Hoechst (fluorescence microscopy) were considered to be in metaphase II (MII) [31]. Oocytes were then rinsed in PBS, frozen in liquid nitrogen, and kept in a minimum volume at −80°C. Cumulus cells were centrifuged (13 000 × *g* for 1 min) in order to obtain a cell pellet. After supernatants were removed, pellets were frozen in liquid nitrogen and stored at −80°C. In order to obtain a mature control for further analysis, 15 COCs collected from preovulatory follicles, of which the cumulus had expanded and the oocyte had reached MII after in vivo maturation, were processed and stored as descried above at collection. To obtain an immature control, 15 COCs from subordinate follicles (diameter <30 mm), of which the cumulus was compact and the oocyte was at the germinal vesicle stage, were processed and stored as described above at collection.

RNA Extraction and Semiquantitative RT-PCR

The Tripure Isolation Reagent Kit (Boehringer-Mannheim, Mannheim, Germany) was used to extract total RNA from oocytes and cumulus cells. This one-step extraction procedure was derived from the method described by Chomczynski and Sacchi [32] and was performed according to the manufacturer's recommendations. Briefly, either a single oocyte (immature or in MII at collection, or MII after culture in the presence of EGF) or pools of two cumulus cell pellets were mixed with 100 μl of tripure reagent and added to 60 μl of chloroform. After centrifugation at 14 000 × *g* for 15 min, RNA in the upper aqueous phase was recovered and precipitated with ethanol and dried. RNA pellets from oocytes and cumulus cells were dissolved in 5 μl and 10 μl of H_2O, respectively, and were reverse transcribed in a final volume of 10 μl and 20 μl, respectively, containing 2 μM of oligo$(dT)_{12\text{-}18}$ (Amersham Pharmacia, Orsay, France), 1.25 mM of each dNTP, 2.5 mM $MgCl_2$, 20 IU of recombinant ribonuclease inhibitor (RNasin), and 100 IU of Moloney murine leukemia virus RT (Promega, Charbonniére, France). The reaction was performed for 60 min at 37°C, then reverse transcriptase was heat-inactivated at 95°C for 5 min.

PCR amplification of IL-1α, IL-1β, IL-1ra, IL-1R1, and IL-1R2 cDNAs was performed with specific oligo-nucleotide pairs (Eurogentec, Nantes, France), the sequences of which are shown in Table 1. Moreover, PCR amplification of β-actin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), two genes expressed constitutively, were used to verify the RNA extraction quality of oocytes and cumulus cells, respectively. The PCR reaction mixture contained 3 μl RT products from oocyte mRNA or 2 μl of RT products from cumulus cell mRNA, 0.5 μM of each sense and antisense primer, 1.5 mM $MgCl_2$, 0.2 μM of each dNTP, 1 μCi of [α-^{33}P]dATP (Amersham), and 1 IU of *Taq* DNA polymerase in 25 μl of the appropriate *Taq* buffer (Promega). PCR reactions were performed in a thermal cycler (GeneAmp PCR system 9700, Perkin Elmer, Norwalk, CT) and were initiated by 3 min of denaturation at 94°C, followed by 38 cycles each consisting of 30 sec at 94°C, 30 sec at primer hybridization temperature (Table 1), and 45 sec at 72°C. PCR was ended by a final elongation step of 3 min at 72°C. PCR efficiency was checked by using a positive control (cDNA from equine macrophages stimulated for 48 h with 10 μg/ml^{-1} of lipopolysaccharides). PCR products were electrophoresed through a 2% agarose gel, stained with 0.2 μg/ml of ethidium bromide, and visualized by ultraviolet illumination. Gels were then dried and exposed to a radiosensitive screen for 10 h in order to detect incorporated radioactivity using a PhosphorImager (STORM; Molecular Dynamics, Sunnyvale, CA). Signals were quantified using image analysis software (ImagQuant; Molecular Dynamics). For oocytes, the relative amount of mRNA of interest was proportional to the intensity of the radioactivity. For cumulus cells, the relative amount of IL-1α, IL-1β, IL-1ra, IL-1R1, and IL-1R2 mRNA was normalized to a constant amount of GAPDH mRNA.

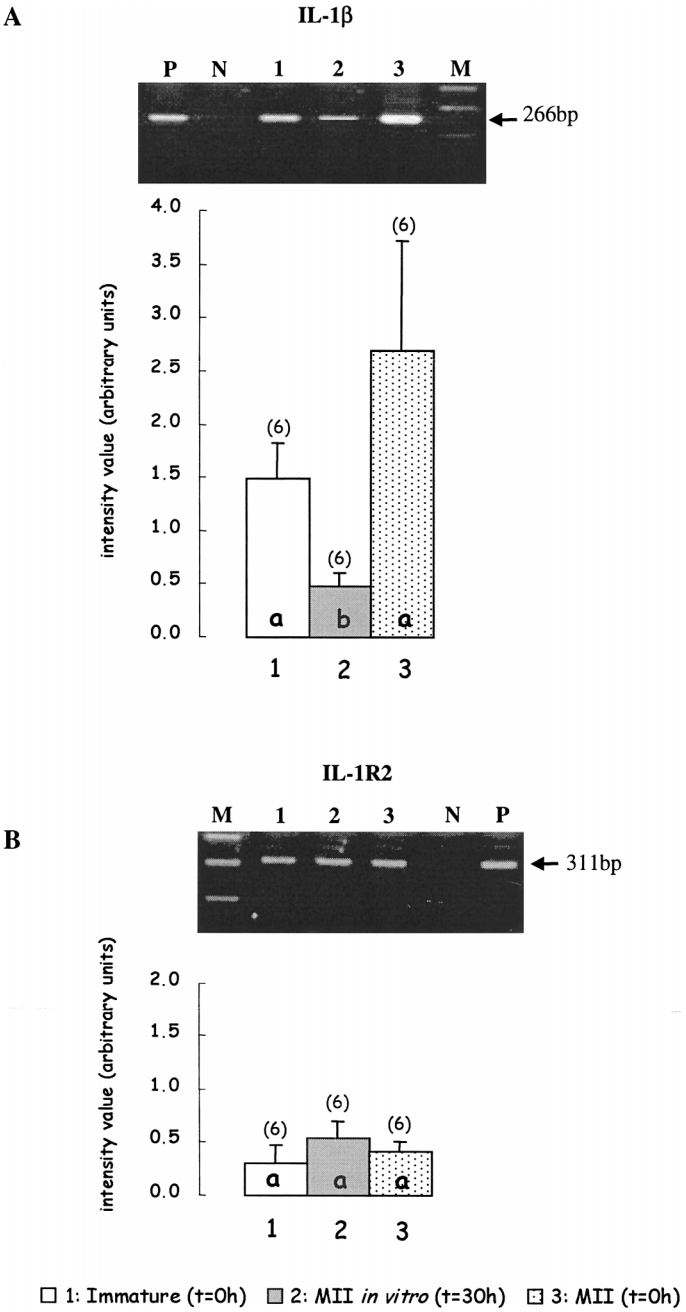

FIG. 1. IL-1β (**A**) and IL-1R2 (**B**) gene expression in equine oocytes at different stages: immature and MII at collection and MII after in vitro culture in medium containing mouse epidermal growth factor. The relative amount of each PCR product isolated by electrophoresis (upper picture) is obtained by scanning each band. Gene expression intensity was measured per oocyte by ImageQuant software and expressed (lower histogram) as an intensity value in arbitrary units. The expected sizes of amplified fragments are indicated. Lane P, positive control of PCR (cDNA from lipopolysaccharide-stimulated equine macrophages); lane N, negative control of PCR (no cDNA); lane M, molecular weight markers (100-bp DNA ladder, Promega). Lane 1, immature oocytes at collection (immature, time = 0 h); lane 2, oocytes that reached MII after culture (MII in vitro, time = 30 h); lane 3, MII oocytes at collection in preovulatory follicles (time = 0 h). The quantification results are expressed in graphs (histogram) as arbitrary units and represent means ± SEM of six oocytes per group. a and b are significantly different ($P < 0.05$) according to the nonparametric Kruskal-Wallis test. The data are representative of three experiments (RNA extraction and PCR).

Statistical Analysis

The chi-square test was used to compare oocyte maturation rates and cumulus expansion rates. The nonparametric Kruskal-Wallis test was performed using StatXact 4 software (CYTEL, Cambridge, MA [www.cytel.com]) in order to compare means of gene expression signals.

RESULTS

To evaluate the involvement of the IL-1 system during COC maturation, the presence of ligands, receptors, and antagonist mRNA was first investigated in oocytes and cumulus cells before and after in vivo and in vitro maturation. Then, the influence of IL-1β on the in vitro oocyte maturation rate and cumulus expansion was studied.

Expression of IL-1 Family Members in Equine Oocytes

Mature (MII) oocytes cultured in the presence of EGF as well as immature (germinal vesicle; GV) and mature oocytes at collection were analyzed by RT-PCR for the presence of IL-1α, IL-1β, IL-1ra, IL-1R1, and IL-1R2 mRNA. Figure 1 shows the results obtained after amplification of IL-1β (Fig. 1A) and IL-1R2 (Fig. 1B) cDNA. An expected 266-base pair (bp) signal was observed for IL-1β in all oocytes regardless of nuclear stage and maturation conditions (in vivo or in vitro; Fig. 1A). Semiquantitative analysis of the signals showed that the IL-1β signal varied between types of oocytes studied, with in vitro-matured oocytes displaying the lowest PCR signal for IL-1β ($P < 0.05$) compared with mature and immature oocytes analyzed at collection. Moreover, IL-1R2 cDNA amplification gave the expected 311-bp fragment in all types of oocytes. The IL-1R2 signal did not vary significantly among the three oocyte types studied (Fig. 1B). By contrast, no signal was observed for other members of the IL-1 system studied (IL-1α, IL-1ra, and IL-1R1), despite the correct amplification of a 212-bp fragment for β-actin mRNA in the samples studied and despite the correct PCR positive control for each target gene (data not shown).

Expression of IL-1 Family Members in Equine Cumulus Cells

Cumulus cells from compact and expanded COCs at collection as well as after in vitro culture in the presence of EGF (medium 3 of experiment 1) were analyzed by semiquantitative RT-PCR for their mRNA content of IL-1 family members, and related to the GAPDH mRNA content. IL-1α mRNA was not detected in any cumulus cells analyzed (data not shown). Specific amplification of IL-1β, IL-1ra, IL-1R1, and IL-1R2 mRNA is illustrated in Figure 2. The amplified fragments were at the correct expected sizes (266 bp, 425 bp, 372 bp, and 311 bp, respectively) and showed variation of intensity between samples. Semiquantitative analysis of PCR products for IL-1β (Fig. 2A) and IL-1R2 (Fig. 2B) showed that the intensity of the signals was low and did not significantly vary in cumulus cells between stages (compact and expanded) nor between culture conditions (in vivo, time = 0 h; in vitro, time = 30 h). However, for both stages studied, signals for IL-1ra (Fig. 2C) and IL-1R1 (Fig. 2D) were higher in cumulus cells analyzed at collection than in those analyzed after in vitro culture. These signals were not different between compact and expanded cumulus cells that were analyzed at collection. After in vitro culture, the IL-1R1 signal was significantly higher in expanded cumulus cells than in compact ones, for which no signal was observed (Fig. 2D).

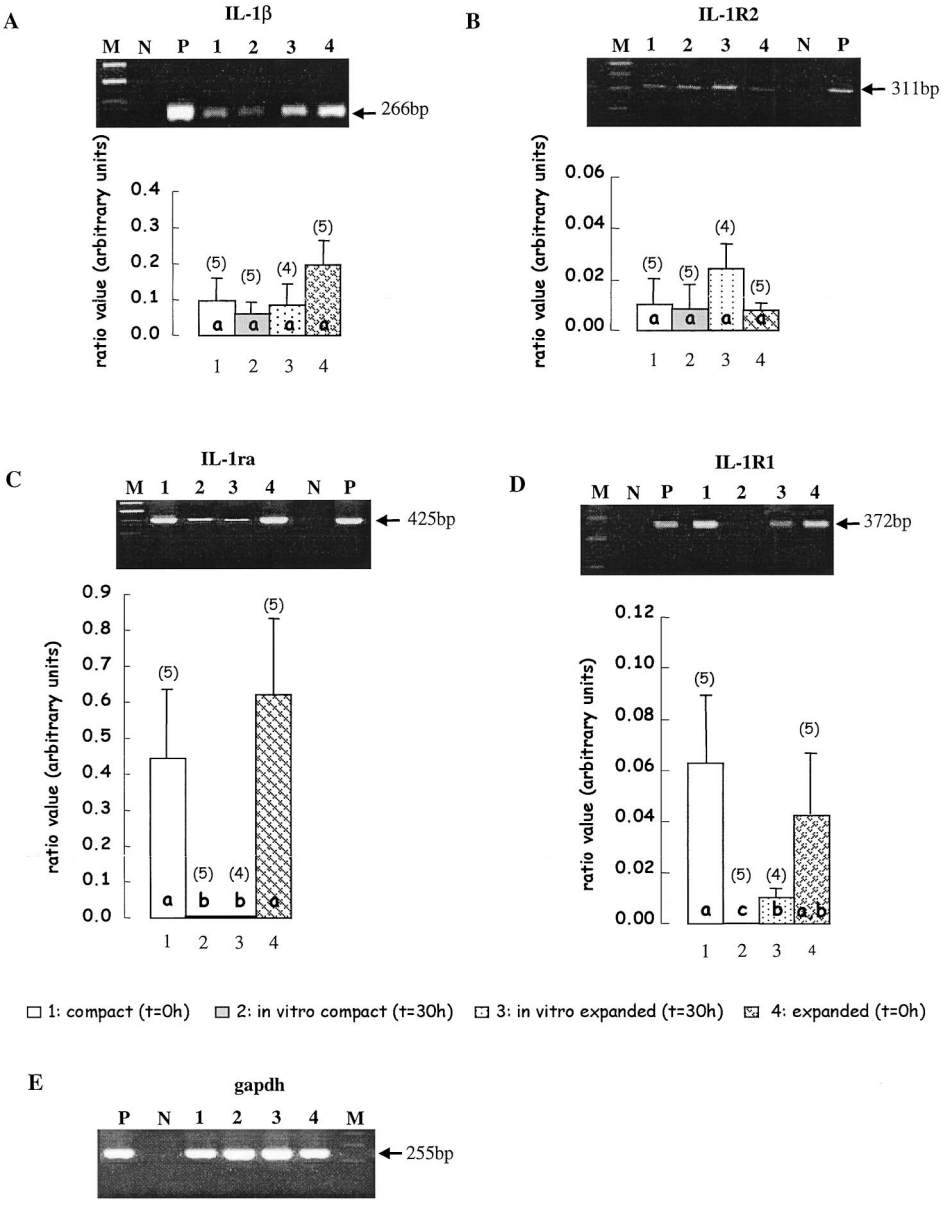

FIG. 2. IL-1 system members (IL-1β, **A**; IL-1R2, **B**; IL-1ra, **C**; IL-1R1, **D**) and GAPDH, **E** gene expression in equine compact (1 and 2) and expanded (3 and 4) cumulus cells at collection (1 and 4) or after in vitro culture (2 and 3) in medium containing mouse epidermal growth factor. The relative amount of each PCR product isolated by electrophoresis (upper picture) is obtained by scanning each band. Gene expression intensity (arbitrary units) was measured per cumulus cell by Image-Quant software and is expressed (lower histogram) as an intensity value normalized to the GAPDH intensity value. The expected size of the fragments is indicated. Lane P, positive control of PCR (cDNA from lipopolysaccharide-stimulated equine macrophages); lane N, negative control of PCR (no cDNA); lane M, molecular weight markers (100-bp DNA ladder, Promega). Lane 1, compact cumulus cells at collection (compact, time = 0 h); lane 2, compact cumulus cells after in vitro culture (in vitro compact, time = 30 h); lane 3, expanded cumulus cells after in vitro culture (in vitro expanded, time = 30 h); lane 4, expanded cumulus cells at collection (expanded, time = 0 h). The results are expressed in histograms as arbitrary units and represent means ± SEM of four or five samples as indicated. a, b, and c are significantly different ($P < 0.05$) according to the nonparametric Kruskal-Wallis test. The data presented are representative of two experiments (RNA extraction and PCR).

In Vitro Effect of IL-1β on Nuclear Maturation of Equine Oocytes

In 2 experiments, 236 COCs were cultured in vitro. As shown in Table 2, the oocyte maturation rate was not different between the basal culture medium (no additive) and the medium supplemented with IL-1β in both experiments. In experiment 1, the oocyte maturation rate was significantly highest in the medium containing EGF. In experiment 2, the oocyte maturation rate observed in medium 4 containing eLH was higher, though not significant, than in medium 1 (no additive; $P < 0.09$). The maturation rate was significantly higher than in medium 2 (IL-1β alone; $P < 0.05$). Furthermore, IL-1β reduced the eLH effect on oocyte maturation (eLH alone vs. eLH + IL-1β; $P < 0.05$). Taken together, these data showed that EGF exhibits and eLH tends to exhibit a positive effect on the nuclear maturation rate, whereas IL-1β alone does not, and rather, it inhibits eLH-induced meiosis resumption in oocytes.

In Vitro Effect of IL-1β on Cumulus Expansion of Equine COCs

Cumulus morphology was analyzed for all 236 cultured COCs. As shown in Table 2, in experiment 1, the cumulu expansion rate was significantly higher in the medium containing EGF than in the basal medium (no additive) or the medium containing IL-1β. The expansion rate was similar in the presence or absence of IL-1β. In experiment 2, the highest cumulus expansion rate after culture was observed in the medium containing eLH in combination with IL-1β, whereas the lowest expansion rate was observed in the basal medium (no additive; $P < 0.05$). Media containing either IL-1β or eLH alone were not significantly different from these media. By cumulating both sets of experimental data, it can be noted that the lowest cumulus expansion rate was observed in the basal medium. The addition of EGF or eLH in combination with IL-1β significantly increased the cumulus expansion rate compared to this basal medium ($P < 0.05$). In contrast to its putative inhibitory effect on nuclear maturation induced by eLH, IL-1β does not seem to down-

TABLE 2. Equine oocyte nuclear maturation rate and cumulus expansion rate after in vitro culture.

	Culture medium* supplemented with	Percentage of oocytes in metaphase II (proportion)†	Percentage of cumulus expansion (proportion)
Experiment 1	M1 (no additive)	34 (14/41)[a]	19 (6/32)[a]
	M2 IL-1β (50 ng ml^{-1})	26 (10/39)[a]	19 (6/32)[a]
	M3 EGF (50 ng ml^{-1})	74 (29/39)[b]	69 (18/26)[b]
Experiment 2	M1 (no additive)	43 (18/42)[a,b]	26 (10/39)[a]
	M2 IL-1β (50 ng ml^{-1})	36 (15/42)[a]	29 (10/35)[a,b]
	M4 eLH (5 μg ml^{-1})	61 (25/41)[b]	43 (15/35)[a,b]
	M5 eLH + IL-1β (5 μg ml^{-1} + 50 ng ml^{-1})	38 (16/42)[a]	46 (17/37)[b]

* Composed of TCM 199 with Earle salt and 0.5% BSA; M, medium; BSA, bovine serum albumin; EGF, epidermal growth factor; IL-1β, interleukin 1 beta; eLH, equine luteinizing hormone.
† Within each experiment, a and b are significantly different ($P < 0.05$).

regulate cumulus expansion induced by eLH. However, the respective expansion rates were not significanty different.

DISCUSSION

The aim of the present work was to analyze IL-1 family gene expression in equine cumulus cells and oocytes, and to investigate the effect of IL-1β in vitro on the maturation rate of oocytes and on cumulus expansion.

In the present study, expression of both IL-1β and IL-1R2 genes was demonstrated for the first time in equine oocytes regardless of maturation stage and conditions. IL-1β gene expression has been previously observed in rat and mouse [33, 34] oocytes, but not in human oocytes [35]. Kol et al. [33, 36] have investigated IL-1R2 and IL-1ra gene expression in rat ovary. In contrast with our results, IL-1R2 transcripts were not detected in rat oocytes, whereas the IL-1ra gene displayed faint expression. However, in accordance with a recent study performed in human oocytes [35], IL-1α, IL-1R1, and IL-1ra transcripts were not found in equine oocytes. Thus, taken together, these observations suggest some species-specific differences in the expression of IL-1 family genes in oocytes from mammals. In equine species, the expression of IL-1R2 mRNA by an oocyte may also suggest that the oocyte is a potential target for IL-1. In our work, there was no quantitative difference between IL-1β and IL-1R2 mRNA levels in immature (GV) and mature (MII) oocytes when analyzed at collection. Considering that in mammalian oocytes gene transcription stops before meiosis and mRNA in stock are used thereafter until the 8- to 16-cell embryo stage, one could hypothesize that IL-1β and IL-1R2 mRNA are mainly translated in oocytes at or after fertilization. Indeed, IL-1 bioactivity has been reported in embryo culture media after the in vitro fertilization of human oocytes [37].

In vitro, equine IL-1R2 gene expression did not vary significantly between the different types of oocytes studied. In contrast, the level of IL-1β transcripts decreased significantly during in vitro maturation. It is interesting that IL-1β levels were significantly higher in preovulatory oocytes that had reached MII in vivo than in oocytes that had reached MII after in vitro culture. To our knowledge, this is the first comparison of IL-1β transcripts between in vivo-matured and in vitro-matured oocytes from mammalian species. The low level of IL-1β transcripts in cultured oocytes could be explained by a decrease in the transcription rate of the gene, a decrease in the maturation of the transcript, an increase in the degradation of the mRNA in vitro, or a combination of these. The low level of IL-1β transcripts after culture could also be explained by the incomplete cytoplasmic maturation of the oocyte. It has already been shown previously that in vitro conditions alter the cytoplasmic maturation quality of equine oocytes [29, 31, 38]. Furthermore, the in vitro situation may not completely reflect the complex stimulations and interactions encountered by oocytes in vivo.

Regarding equine cumulus cells, the present work has reported IL-1β, IL-1ra, IL-1R1, and IL-1R2 gene transcription. This is in agreement with results obtained in humans [35] and rats [33, 36] demonstrating that, in these species, cumulus cells express IL-1β, IL-1R1, and IL-1ra, whereas IL-1R2 has not been studied in humans and has not been detected in rats. To our knowledge, no data on the expression of these IL-1 family genes in cumulus cells are available in other mammalian species. From in vitro to in vivo and between stages of maturation, IL-1β and IL-1R2 mRNA levels did not vary significantly in equine cumulus cells. In contrast, IL-1ra and IL-1R1 mRNA levels decreased significantly after the culture period. In our model, a high level of expression of IL-1ra and IL-1R1 genes in vivo in cumulus cells could be noted regardless of the stage of maturation, and they were only faintly or not at all detectable after in vitro culture conditions. Once again, the in vitro conditions may not be suitable to maintain the expression level of IL-1ra and IL-1R1 genes in cumulus cells compared to in vivo conditions. Despite the faint expression of IL-1ra and IL-1R1 genes in vitro, cumulus expansion was observed, suggesting that these two IL-1 family members do not participate in the molecular process of cumulus expansion.

Overall, our results have specified the expression site of IL-1 system genes at the equine COC level. Our results indicate that IL-1β and one of its receptors, IL-1R2, are expressed in the oocytes and the entire IL-1 family, but that IL-1α is expressed in cumulus cells. These observations suggest that IL-1 family members are involved in autocrine interaction, paracrine interaction, or both, in cumulus-oocytes complexes, as was shown for TNFα in preovulatory ovine follicles [39]. Cumulus cells, by producing IL-1ra, could exert feedback control on the IL-1β-mediated inflammatory process involved in maturation. Nevertheless, the effect of IL-1 at the oocyte and cumulus levels remains unclear, whereas the central role of IL-1 in the ovarian function, especially in the modulation of steroidogenesis [19, 21, 40], regulation of prostaglandin [41] and protease [14, 16] synthesis, and directly in the ovulatory process [11, 12], is well documented. The few studies describing the IL-1 influence on oocyte maturation are contradictory. Indeed, IL-1β was shown to be unable to stimulate germinal vesicle breakdown in rat models [11], in contrast to rabbit [12] and bovine [42] models.

The second part of the present work was designed to study the in vitro effect of exogenous IL-1β on the maturation of equine COCs. As expected (for a review see [43]), the addition of eLH to the standard culture medium tended to increase the oocyte nuclear maturation rate. The effect of LH on COCs is mediated by the binding of LH on receptors whose presence on cumulus cells has been previously demonstrated in equine species [44]. The addition of IL-1β remained without effect when added alone to the basal medium. This result could be related to the study performed on perfused rat ovary showing that IL-1β induces ovulation but not the germinal vesicle breakdown of oocytes [11]. It is worth noting that in the presence of eLH, IL-1β decreased the oocyte maturation rate to the rate observed with no additive medium. Therefore, IL-1β is able to reverse or block the action of LH on oocyte meiosis resumption. This could be explained either by the direct action of IL-1β on LH or its receptor or an indirect effect by inducing the synthesis of an inhibitory factor. This effect could either be on the oocyte itself or mediated by cumulus cells. The inhibitory effect of IL-1β on LH-induced oocyte nuclear maturation could appear confusing in view of the presence of IL-1β mRNA in immature and mature oocytes. Nevertheless, as mentioned above, oocytes are characterized by mRNA in stock until their translation and use after fertilization. One can hypothesize that IL-1β mRNA is one of these in-stock mRNAs. Thus, the intraoocyte IL-1β would not be involved in the regulation of LH-induced oocyte nuclear maturation, as observed in vitro when exogenous IL-1β is added to the culture medium. In conclusion, this study demonstrated the expression of IL-1β and IL-1R2 genes in equine oocytes and the expression of IL-1β, IL-1ra, IL-1R1, and IL-1R2 genes in equine cumulus cells. We clearly showed the inhibitory effect of IL-1β on eLH-induced meiosis resumption of equine oocytes without reducing cumulus cell expansion. IL-1β thus seems to display a selective cellular effect. Although the mechanism by which IL-1β regulates oocyte maturation is unclear, the IL-1 family may play an essential role in the physiology of cumulus-oocyte complexes in the equine species.

ACKNOWLEDGMENTS

We thank Isabelle Couty, Guy Duchamp, and the staff of the experimental stud division for technical assistance. Equine luteinizing hormone (eLH) was generously provided by the National Hormone and Pituitary Program of the National Institute for Diabetes and Digestive and Kidney Diseases (A.F. Parlow, National Institutes of Health). The authors thank Anne-Marie Wall for assistance with English correction.

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