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Presence and regulation of messenger ribonucleic acids encoding components of the class II major histocompatibility complex-associated antigen processing pathway in the bovine corpus luteum

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Abstract

Luteal cells express class II major histocompatibility complex (MHC) molecules and can stimulate T lymphocyte proliferation *in vitro*. However, it is unknown whether luteal cells express the intracellular components necessary to process the peptides presented by class II MHC molecules. The objective of the present study was to examine the expression and regulation of three major class II-associated antigen processing components – class II MHC-associated invariant chain (Ii), DMα and DMβ – in luteal tissue. Corpora lutea were collected early in the estrous cycle, during midcycle and late in the estrous cycle, and at various times following administration of a luteolytic dose of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) to the cow. Northern analysis revealed the presence of mRNA encoding each of the class II MHC-associated antigen processing proteins in luteal tissue. Ii mRNA concentrations did not change during the estrous cycle, whereas DMα and DMβ mRNA concentrations were highest in midcycle luteal tissue compared with either early or late luteal tissue. Tumor necrosis factor-α (TNF-α) reduced DMα mRNA concentrations in cultured luteal cells in the presence of LH or $PGF_{2\alpha}$. DMα and DMβ mRNA were also present in highly enriched cultures of luteal endothelial (CLENDO) cells, and DMα mRNA concentrations were greater in CLENDO cultures compared with mixed luteal cell cultures. Expression of invariant chain, DMα and DMβ genes indicates that cells within the corpus luteum express the minimal requirements to act as functional antigen-presenting cells, and the observation that CLENDO cells are a source of DMα and DMβ mRNA indicates that non-immune cells within the corpus luteum may function as antigen-presenting cells.

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Introduction

Luteal regression is a complex cascade of events, initiated by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), that causes cessation of luteal function and subsequent structural demise of the corpus luteum (CL). Several lines of evidence suggest participation of the immune system in this process. Macrophages and T lymphocytes are present in the CL of several species, and infiltrate the CL near the time of luteal regression and in response to $PGF_{2\alpha}$ (Bagavandoss *et al.* 1990, Brännström *et al.* 1994, Lawler *et al.* 1999, Penny *et al.* 1999, Krusche *et al.* 2002, Townson *et al.* 2002, Komatsu *et al.* 2003, Neuvians *et al.* 2004). Cytokines produced by these cells – including tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-1β and IL-6 – decrease progesterone production, increase prostaglandin synthesis and stimulate apoptotic death in cultured luteal cells (Nothnick & Pate 1990, Fairchild & Pate 1991, Benyo & Pate 1992, Gorospe *et al.* 1992, Townson & Pate 1994, 1996, Jo *et al.* 1995, Del Vecchio & Sutherland 1997, Young *et al.* 1997, Petroff *et al.* 2001). The presence of these cytokines has been detected in luteal tissue (Bagavandoss *et al.* 1990, Ji *et al.* 1991, Penny *et al.* 1999, Petroff *et al.* 1999, Krusche *et al.* 2002, Townson *et al.* 2002, Komatsu *et al.* 2003, Neuvians *et al.* 2004), indicating that macrophages and T cells present within the CL are activated. Class II major histocompatibility complex (MHC) molecules are also expressed by luteal cells of several species (Khoury & Marshall 1990, Benyo *et al.* 1991, Kenny *et al.* 1991, Penny *et al.* 1999),

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providing a means of activation of T lymphocytes present in luteal tissue.

Class II MHC molecules, which are typically expressed on antigen-presenting cells of the immune system (i.e. macrophages, dendritic cells and B lymphocytes), present small peptides processed intracellularly to T lymphocytes (Sant 1994). When cells expressing class II MHC molecules (antigen-presenting cells) interact with T cells, the peptides bound to the MHC molecules determine whether the antigen-presenting cell will activate T cells. The immune system is tolerant of normally expressed self-peptides, but T lymphocytes can be activated by cells expressing peptides derived from a foreign source (i.e. viral or bacterial peptides) as well as self-peptides that are presented by MHC molecules under abnormal conditions (i.e. tumor antigens). As class II MHC molecules are synthesized, they immediately bind to a chaperone protein called invariant chain (Ii), a portion of which occupies the peptide-binding groove of the MHC molecule (Roche & Cresswell 1990, Avva & Cresswell 1994). Invariant chain serves a dual function, directing intracellular transport of class II MHC molecules (Bakke & Dobberstein 1990, Lotteau *et al.* 1990) and ensuring that binding of 'inappropriate' peptides does not occur prematurely (Roche & Cresswell 1990). Class II MHC–Ii complexes are transported into specialized intracellular compartments in which proteases degrade the Ii into a peptide called class II-associated invariant chain peptide (CLIP) (Maric *et al.* 1994, Riese *et al.* 1996). Prior to binding of antigenic peptides by class II MHC molecules, the CLIP fragment bound to the peptide binding groove must be removed. Removal of CLIP is carried out by a class II MHC-like heterodimeric protein referred to as DM (Fling *et al.* 1994, Morris *et al.* 1994). Following CLIP removal, DM stabilizes class II MHC molecules to allow efficient binding of antigenic peptides (Denzin *et al.* 1996).

In addition to the primary roles for Ii and DM described above, these intracellular proteins have been implicated in a secondary role as 'editors' of the repertoire of peptides presented to T cells in the context of class II MHC, since they control in part the type of peptides that bind to class II MHC molecules and that are presented to T lymphocytes (Castellino *et al.* 1997, Vogt *et al.* 1999). Antigen-presenting cells taken from Ii or DM knockout animals stimulate activation and proliferation of otherwise non-reactive T cell clones and reveal the presence of 'self-reactive' T cells in normal animals (Bodmer *et al.* 1994, Katz *et al.* 1996, Marieke van Ham *et al.* 1996, Lightstone *et al.* 1997), indicating that alterations in Ii or DM expression may alter the ability of antigen-presenting cells to stimulate T cell activation.

Expression of class II MHC molecules is not limited to cells of the immune system, and in many cases expression of class II MHC molecules by other cell types is associated with inflammation and/or autoimmune disorders. Cells of the bovine CL express class II MHC molecules, and class II MHC expression increases near the end of the estrous cycle and in response to a luteolytic dose of $PGF_{2\alpha}$ (Benyo *et al.* 1991). Bovine luteal cells also stimulate class II MHC-dependent *in vitro* proliferation of T cells isolated from the same animal (autologous T cells) (Petroff *et al.* 1997). Following administration of a luteolytic dose of $PGF_{2\alpha}$ to the cow, the ability of luteal cells to stimulate T lymphocyte proliferation is enhanced (Petroff *et al.* 1997). The enhancement of luteal cell-stimulated T lymphocyte proliferation may be due at least in part to the effect of $PGF_{2\alpha}$ on progesterone synthesis *in vitro* (Cannon *et al.* 2003), which decreases following *in vivo* exposure to $PGF_{2\alpha}$ (Pate & Nephew 1988). However, we speculate that changes in expression of class II MHC genes and associated antigen-processing genes may also be involved in the enhancement of luteal cell-stimulated T cell proliferation, and that processing of antigens by luteal cells may be altered near the time of luteal regression. Alteration of antigen processing within luteal cells could result in binding of a different repertoire of peptides to class II MHC molecules, allowing luteal cells to stimulate T cell activation during luteal regression. The hypothesis tested in the present study is that expression of either Ii or DM changes near the end of the estrous cycle. Due to observations in the initial portion of the present study, we wished to determine whether steady-state concentrations of DMα and DMβ mRNA are regulated by luteinizing hormone (LH) and $PGF_{2\alpha}$, the classic hormonal regulators of luteal function, or by TNF-α, which is suspected to play a significant role in regulation of luteal function. We have also examined highly enriched cultures of luteal endothelial (CLENDO) cells for the presence of DMα and DMβ mRNA, to determine whether endothelial cells of the CL express the components of the class II MHC antigen-processing pathway.

Materials and Methods

Reagents

Powdered Ham's F-12 culture medium, gentamicin, fetal bovine serum, *Escherichia coli* DH5α chemically competent cells, and TRIzol Reagent were all purchased from Life Technologies (Grand Island, NY, USA). Recombinant murine TNF-α was purchased from Life Technologies and R&D Systems (Minneapolis, MN, USA). The National Hormone and Pituitary Program (Baltimore, MD, USA) provided bovine LH (NIAMMD-bLH-4). Insulin-transferrin-selenium (ITS) premix was obtained from BD Biosciences (Bedford, MA, USA). Bovine serum albumin (fraction V), $PGF_{2\alpha}$, HEPES buffer and Bouin's fixative were purchased from Sigma. Prefer fixative was purchased from Anatech Ltd (Battle Creek, MI, USA). Type I collagenase was acquired from Worthington Biochemical Corp. (Freehold, NJ, USA). SDS and MOPS buffer were acquired from Amresco (Solon, OH, USA). Digoxigenin-labeled rNTP mix, alkaline-phosphatase-conjugated anti-digoxigenin Fab fragments, CDP-Star chemiluminescent substrate, blocking

reagent, LightCycler-FastStart DNA SYBR Green I kits and ampicillin were all purchased from Roche. Random hexamer primers were purchased from Amersham. SuperScript II reverse transcriptase was acquired from Invitrogen. DyNAmo HS SYBR Green qPCR kits were purchased from MJ Research (Boston, MA, USA). Restriction enzymes, RNA polymerases, dNTPs, recombinant RNase inhibitor, Moloney murine leukemia virus (M-MLV) reverse transcriptase, isopropyl-β-D-thiogalactopyranoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) were purchased from Promega. Hybond N + membranes were obtained from Amersham Pharmacia Biotech (Piscataway, NJ, USA). Tissue culture flasks were from Corning (Acton, MA, USA). All other chemicals were purchased from Sigma or Fisher Scientific (Fairlawn, NJ, USA).

Animals and tissue collection

Multiparous, lactating dairy cows between 3 and 6 years of age were used in the present studies. Animals were housed indoors and had complete freedom of movement; they were given regular access to a total mixed ration and were allowed to feed *ad libitum*. Corpora lutea were removed transvaginally from regularly cycling dairy cows. Blood samples were collected from all animals prior to CL removal to determine circulating concentrations of progesterone. Corpora lutea were cut into four equal pieces, three of which were immediately snap frozen in liquid nitrogen, transported to the laboratory and stored at −80 °C until RNA extraction was performed. In the first experiment, CL were removed early in the estrous cycle (day 5, $n = 6$), during midcycle (days 9–12, $n = 6$) or late in the estrous cycle (day 18, $n = 6$), or at 0.5 ($n = 5$), 1 ($n = 5$), 4 ($n = 5$), 12 ($n = 4$) or 24 h ($n = 5$) following i.m. administration of 25 mg $PGF_{2\alpha}$ (Lutalyse; Upjohn Co., Kalamazoo, MI, USA). In the second experiment, CL were removed during midcycle, immediately placed in ice-cold Ham's F-12 culture medium on ice and transported to the laboratory for dissociation. Handling of animals and surgical procedures were carried out in accordance with protocols approved by the Institutional Laboratory Animal Care and Use Committee of The Ohio State University.

RNA isolation and Northern analysis

Frozen tissue collected for the first experiment was homogenized in TRIzol reagent using a Polytron tissue homogenizer (Brinkman Instruments, Westbury, NY, USA). In the second experiment, RNA from cultured cells was isolated by adding TRIzol reagent directly to culture flasks. Following tissue homogenization or cell lysis, total cellular RNA was isolated according to procedures outlined by the manufacturer. The final RNA precipitate was resuspended in double-distilled water containing 0.1% diethyl pyrocarbonate (DEPC) and RNA concentration was determined spectrophotometrically.

Fifteen micrograms of total cellular RNA were electrophoretically separated on 1.5% agarose denaturing gels containing 20 mM MOPS buffer, 5 mM sodium acetate, 1 mM EDTA and 0.66 M formaldehyde. Following electrophoresis, gels were stained for 15 min with 1 μg/ml ethidium bromide in DEPC-treated water, destained for 1 h in DEPC-treated water and photographed. RNA was transferred to Hybond-N + membranes, and membranes were baked at 80 °C for 2 h to crosslink RNA to the membranes. Gels were restained with eithidum bromide following transfer and examined under u.v. light to confirm complete transfer of RNA.

Digoxigenin-labeled riboprobes were synthesized using plasmids containing full-length Bovine Lymphocyte Antigen (BoLA)-DMα, BoLA-DMβ or bovine class II MHC-associated Ii cDNA sequences. Plasmids containing these sequences have been described previously (Niimi *et al.* 1995, 1996) and were provided by Dr Yoko Aida (The Institute of Physical and Chemical Research, Tsukuba, Japan). Digoxigenin-labeled riboprobes were generated using linearized plasmids according to the instructions in Roche Molecular Biochemicals' *DIG Nonradioactive Nucleic Acid Labeling and Detection System* manual. Membranes were prehybridized at 68 °C for 1 h with hybridization core buffer (250 mM sodium phosphate, 1 mM EDTA, 5% (w/v) SDS containing 0.5% (w/v) Roche DIG Nucleic Acid Detection System Blocking Reagent), and hybridization was carried out for 16 h at 68 °C. Following hybridization, membranes were washed and detection was performed using alkaline phophatase-conjugated anti-digoxigenin Fab fragments in concert with the chemiluminescent CDP-Star substrate, according to the specifications in Roche Molecular Biochemical's *DIG Nonradioactive Nucleic Acid Labeling and Detection System* manual. Membranes were exposed to Biomax ML film (Eastman Kodak Co., Rochester, NY, USA) to detect chemiluminescence.

Quantification of steady-state concentrations of each message was performed densitometrically using a PDI 420oe scanning densitometer. The densitometric value (in arbitrary densitometric units) of the band of interest in each sample was standardized to the densitometric values of the corresponding 18S rRNA.

Luteal cell dissociation and culture

Dissociation of luteal cells was carried out according to procedures described previously (Pate & Condon 1982). Cell culture was performed in a humidified atmosphere of 5% CO_2 in air at 37 °C. Dispersed luteal cells (4×10^6 cells/flask) were cultured in Ham's F-12 medium supplemented with insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml), gentamicin (20 μg/ml) and LH (1 ng/ml) in serum-coated 25 cm^2 flasks, in a total of 4 ml of culture medium. Cells were allowed to adhere overnight, medium was replaced and cultures were treated with LH (10 ng/ml), TNF-α (50 ng/ml) or $PGF_{2\alpha}$ (10 ng/ml) in a

3 × 3 factorial arrangement. Treatment concentrations used in this study have been shown previously to affect function, viability and gene expression in cultured bovine luteal cells (Pate & Condon 1984, Townson & Pate 1994, Petroff *et al.* 2001, Cannon & Pate 2003). Medium and treatments were replaced after 24 h. Total RNA was extracted from cultured cells after 48 h of culture. The experiment was replicated a total of four times using CL from different animals.

Isolation and culture of purified luteal endothelial cells

Purified endothelial cells from bovine CL (CLENDO cells) collected during early pregnancy were purchased from Cambrex BioScience (BioWittaker, Walkersville, MD, USA) as described previously (Cavicchio *et al.* 2002, Pru *et al.* 2003, Liptak *et al.* 2005). In the present study, endothelial cells from frozen aliquots (passages 3–5; 5000 cells/cm^2) were cultured in EGM-2MV media, as recommended by the supplier with 3% fetal bovine serum (Cambrex Bioscience) in 60 mm dishes. Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Culture medium was replaced every 48 h until 80–90% confluent. On the day of harvest, cells were equilibrated for 2–3 h in serum-free EBM-2 medium. CLENDO cells were collected in RLT lysis buffer and RNA was isolated using the RNeasy kit (Qiagen, Valencia, CA, USA) according to the manufacturer's specifications.

Quantitative reverse transcription-PCR (RT-qPCR)

Primers used to amplify DMα and DMβ cDNAs were designed based on the published sequences for DMα and DMβ (Niimi *et al.* 1995; GenBank accession numbers D76416 and D76417 respectively). Primer sequences were 5′-GCTCTCTGAGTCCTACGACGA-3′ and 5′-GCACAGCACATTCTCCAGAAG-3′ for DMα forward and reverse primers respectively, and 5′-CACCTGTGTGTTGGACGATGA-3′ and 5′-AGCGTTACCAGAGCCACAGAA-3′ for DMβ forward and reverse primers respectively. Amplification using DMα primers resulted in a single 526 bp DMα cDNA fragment, whereas amplification using DMβ primers resulted in a single 597 bp DMβ cDNA fragment. Primer sequences used for amplification of steroidogenic acute regulatory protein (StAR) and CD31 (an endothelial cell adhesion molecule) mRNA in cultures of mixed luteal cells or luteal endothelial cells were based on the published sequences (Hartung *et al.* 1995, GenBank accession number NM_174189; Stewart *et al.* 1996, GenBank accession number U35433) and were as follows: 5′-CCTCTCTACAGCGACCAA-3′ and 5′-TCGTGAGTGATGACCGTG-3′ for StAR forward and reverse primers respectively; 5′-GTTCAGCGAAGTTCTGCGAG-3′ and 5′-CTTGCTGGCTGTGGTCTTGT-3′ for CD31 forward and reverse primers respectively. Amplification using the CD31 primers resulted in a 229 bp CD31 cDNA product and amplification using StAR primers yielded a 311 bp StAR cDNA product. The identity of all PCR products was confirmed by DNA sequence analysis. An 854 bp glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA fragment was amplified in parallel reactions, using ovine specific primers described previously (Tsai *et al.* 1996), and concentrations of DMα, DMβ, CD31 and StAR in each sample were standardized to the corresponding G3PDH concentrations.

Steady-state concentrations of mRNA encoding StAR, CD31, DMα and DMβ were determined in total RNA extracted from cultures of mixed luteal cells and luteal endothelial cells, using an MJ Research Opticon 2 real-time PCR thermal cycler. Prior to PCR, reverse transcription using random hexamer primers was performed on 2 μg of total RNA extracted from cultures. PCR was then performed, using 200 ng of reverse transcribed cDNA, using the DyNAmo HS SYBR Green qPCR kit according to the manufacturer's instructions. Thermal cycling was carried out using the following conditions: denaturation, 94 °C for 30 s; annealing, 56 °C for 30 s; extension, 72 °C for 60 s, for a total of 32 cycles. Melting curve analysis was performed after the end of the last cycle and, in conjunction with gel electrophoresis of amplified products, was used to verify the amplification of a single product in each sample. Fluorescence values in each tube were measured at the end of each cycle using single acquisition mode. Fluorescence values of the product of interest in each sample were standardized to the corresponding G3PDH fluorescence values, and these standardized values were then used to calculate the mean steady-state amounts of each message. Since no standard curve was run for the messages of interest, values were expressed in arbitrary units of fluorescence for the purposes of analysis.

Steady-state concentrations of DMα and DMβ mRNA were determined in total RNA extracted from luteal cells treated with LH, $PGF_{2\alpha}$ and TNF-α using a Roche LightCycler real-time PCR thermal cycler. Prior to quantitative PCR, DMα, DMβ or G3PDH reverse primer was used to reverse transcribe 200 ng of total cellular RNA extracted from cultured cells. Quantitative PCR was then performed on 40 ng of reverse transcribed RNA, using the Roche LightCycler-FastStart DNA Master SYBR Green I kit according to the manufacturer's instructions. As a standard curve, DMα, DMβ and G3PDH cDNAs were amplified from lymph node total RNA, electrophoretically separated on a 1.2% agarose gel and eluted. The concentration of purified cDNA products was determined spectrophotometrically, and serial dilutions were prepared and used as an external standard curve in the quantitative PCR assays. Thermal cycling was carried out using the following conditions: denaturation, 94 °C for 30 s; annealing, 58 °C for 30 s; extension, 72 °C for 60 s, 40 cycles total. The ramping speed for transition from denaturation to annealing steps and annealing to extension steps was set manually at 1°/s, while the ramping speed from extension to denaturation steps was set at 20°/s to minimize total running time. Melting curve analysis was performed after the end of the last cycle in order to verify the amplification of a single

product in each sample. There was no evidence of primer dimers or bands other than the desired amplicons in the reactions as determined by melting curve analysis, and this was confirmed by gel electrophoresis of amplified products. The lack of undesired products allowed fluorescence measurements to be taken at the same temperature (72 °C) at which extension was performed. Fluorescence values in each tube were measured at the end of each cycle using single acquisition mode. Samples were run in duplicate and fluorescence values for duplicate samples were averaged. The baseline fluorescence reading and noise band cut-off were set manually for samples and standards, in order to eliminate background fluorescence values from calculation of the slope and *y*-intercept of the standard curve. A standard curve was generated using average fluorescence values of duplicate standards. Average fluorescence values of samples were then used to calculate the concentration of target cDNA in each amplification reaction, using the Fit Points methods of the accompanying LightCycler data analysis software.

Statistical analysis

Normalized densitometric values collected from Northern analyses in the first experiment were subjected to two-way analysis of variance to determine whether differences in mean densitometric values of DMα, DMβ, or Ii RNA existed between stages of the estrous cycle. In the instance of a significant *F* value, the Student–Newman–Kuels (S–N–K) test was used to determine differences between specific means. Relative differences in steady-state concentrations of CD31, StAR, DMα and DMβ mRNA between cultures of mixed luteal cells or luteal endothelial cells were determined using Student's *t* test. In the third experiment, steady-state concentrations of DMα or DMβ mRNA in treated luteal cell cultures were subjected to one-way ANOVA to determine if differences existed between treatment means. In the incidence of a significant *F* value, the S–N–K test was used to determine differences between specific treatment means. Differences were considered to be significant at $P < 0.05$. A *P* value between 0.05 and 0.08 was considered to show a tendency towards a significant difference. All statistical procedures were performed using the SigmaStat statistical analysis software package (Jandel Corporation, San Rafael, CA, USA).

Results

The results of the Northern analyses to detect and quantify Ii, DMα and DMβ mRNA in luteal tissue are displayed in Figs 1 to 3. Figure 1A demonstrates the presence of an Ii transcript of approximately 1.3 Kb in all luteal tissue samples. Muscle tissue, which served as a source of negative control RNA, was devoid of any corresponding band (not shown). There was a large amount of variability

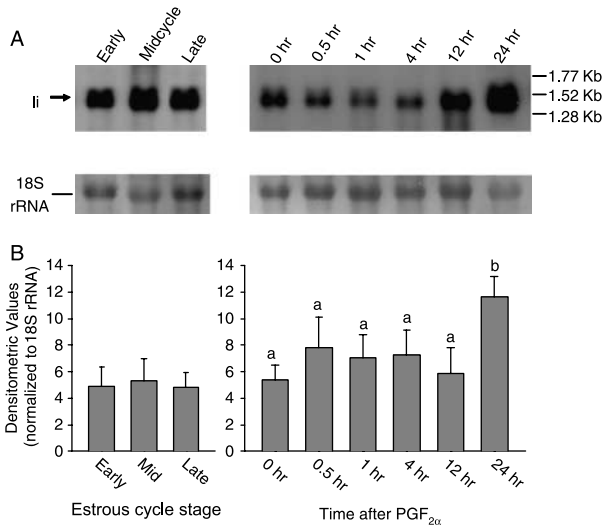

Figure 1 Northern analysis of Ii mRNA in bovine luteal tissue. (A) Northern blot showing Ii mRNA in a single CL collected from each of the following times of the estrous cycle: early (day 5, $n = 6$); during midcycle (days 10–12, $n = 6$); or late (day 18, $n = 6$); and at 0 ($n = 5$), 0.5 ($n = 5$), 1 ($n = 5$), 4 ($n = 5$), 12 ($n = 4$) or 24 h ($n = 5$) following administration of 25 mg $PGF_{2\alpha}$ to the cow. 18S rRNA corresponding to each sample is also shown. (B) Steady-state concentration of Ii mRNA in luteal tissues collected at each time point (mean±S.E.M., *n* values are as stated above). Bars represent densitometric values of Ii mRNA standardized to 18S rRNA. Values with different letters are significantly different ($P < 0.05$).

among animals in the steady-state concentration of Ii mRNA following *in vivo* administration of $PGF_{2\alpha}$. This is responsible for the apparent discrepancy between the density of bands in the Northern blot shown in Fig. 1A and the graph shown in Fig. 1B, which represents the mean of data from four or five animals at each time after $PGF_{2\alpha}$ administration. There were no differences in concentrations of Ii mRNA between CL collected at any stage of the estrous cycle; however there was a greater than two-fold increase in steady-state concentrations of Ii mRNA in CL collected 24 h after $PGF_{2\alpha}$ administration ($P < 0.05$, Fig. 1B). Figure 2A shows a Northern blot of DMα mRNA demonstrating the presence of a single DMα transcript in luteal tissue. Muscle tissue RNA was devoid of any corresponding band (not shown). Similar to the results of Ii Northern analysis, a large amount of variability among animals was also found in DMα mRNA concentrations following $PGF_{2\alpha}$ administration, which is responsible for the apparent difference between the density of bands in the Northern blot shown in Fig. 2A and the graph shown in Fig. 2B, which represents the mean of data from four or five animals at each time after $PGF_{2\alpha}$ administration. Such variability was not observed among animals in CL collected during the estrous cycle. Concentrations of DMα mRNA tended ($P > 0.08$) to be greater in CL collected during midcycle compared with CL collected early (day 5) or late (day 18) in the estrous cycle (Fig. 2B). There were no differences ($P > 0.10$) in concentrations of

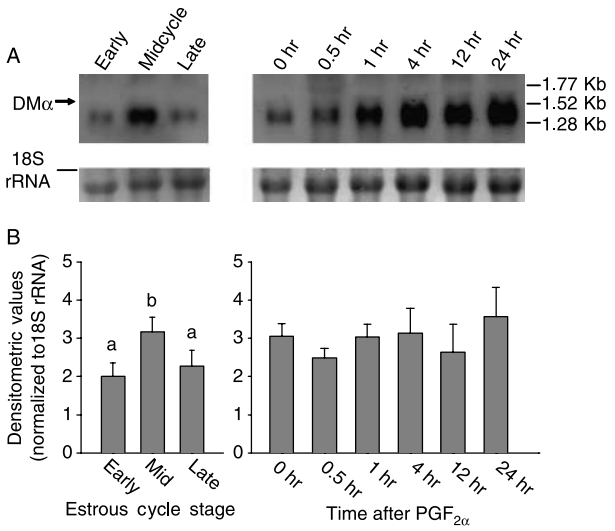

Figure 2 Northern analysis of DMα mRNA in bovine luteal tissue. (A) Northern blot showing DMα mRNA in a single CL collected from each of the following times of the estrous cycle: early (day 5, $n = 6$); during midcycle (days 10–12, $n = 6$); or late (day 18, $n = 6$); and at 0 ($n = 5$), 0.5 ($n = 5$), 1 ($n = 5$), 4 ($n = 5$), 12 ($n = 4$) or 24 h ($n = 5$) following administration of 25 mg $PGF_{2\alpha}$ to the cow. 18S rRNA corresponding to each sample is also shown. (B) Steady-state concentrations of DMα mRNA in luteal tissues collected at each time point (mean±S.E.M., n values are as stated above). Bars represent densitometric values of DMα mRNA standardized to 18S rRNA. Values with different letters tended to be significantly different ($P < 0.08$).

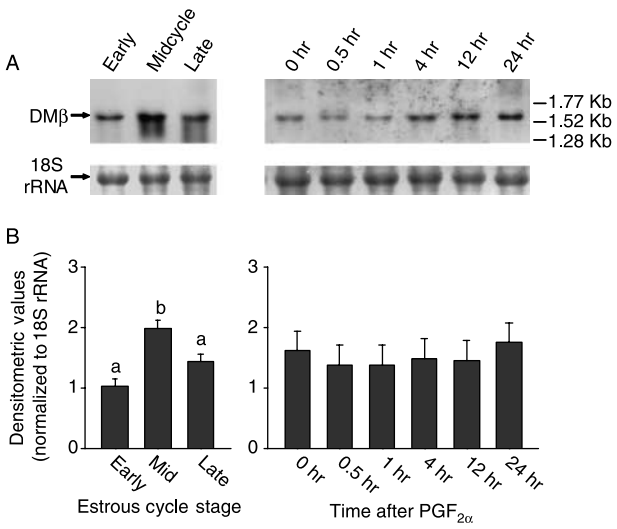

Figure 3 Northern analysis of DMβ mRNA in bovine luteal tissue. (A) Northern blot showing DMβ mRNA in a single CL collected from each of the following times of the estrous cycle: early (day 5, $n = 6$); during midcycle (days 10–12, $n = 6$); or late (day 18, $n = 6$); and at 0 ($n = 5$), 0.5 ($n = 5$), 1 ($n = 5$), 4 ($n = 5$), 12 ($n = 4$) or 24 h ($n = 5$) following administration of 25 mg $PGF_{2\alpha}$ to the cow. 18S rRNA corresponding to each sample is also shown. (B) Steady-state concentrations of DMβ mRNA in luteal tissues collected at each time point (mean±S.E.M., n values are as stated above). Bars represent densitometric values of DMα mRNA standardized to 18S rRNA. Values with different letters are significantly different ($P < 0.05$).

DMα mRNA in CL collected following administration of a luteolytic dose of $PGF_{2\alpha}$, possibly due to the large degree of animal-to-animal variation mentioned earlier. Messenger RNA encoding DMβ was also detected in luteal tissue by Northern analysis, as demonstrated by the presence of a single DMβ transcript in all luteal tissue samples (Fig. 3A). Muscle tissue RNA was also devoid of any band corresponding to DMβ (not shown). Concentrations of DMβ mRNA were greater ($P < 0.05$) in midcycle CL as compared with CL collected early or late in the estrous cycle (Fig. 3B), similar to DMα. There were no differences in concentrations of DMβ mRNA following $PGF_{2\alpha}$ administration.

Steady-state concentrations of DMα and DMβ mRNA in CLENDO cells and mixed luteal cells were compared in order to determine whether endothelial cells derived from the bovine corpus luteum expressed DMα and DMβ. Purity and composition of the CLENDO cell cultures and mixed luteal cell cultures was assessed using quantitative RT-PCR to determine steady-state concentrations of mRNA encoding CD31 and StAR protein. CD31 mRNA was present both in mixed luteal cell and CLENDO cultures, with much greater steady-state concentrations present in CLENDO cultures. StAR mRNA, while abundant in mixed luteal cell cultures, was undetectable in some CLENDO cultures, and was present in concentrations near the detection limit of the PCR assay in others (data not shown). Messenger RNA encoding DMα and DMβ was detectable in cultures of mixed luteal cells as well as CLENDO cell cultures. Steady-state concentrations of DMα, but not DMβ, mRNA were greater in CLENDO cultures (Fig. 4).

The effects of LH, $PGF_{2\alpha}$ and TNF-α on the concentrations of DMα mRNA in cultured bovine luteal cells are displayed in Fig. 5. Concentrations of DMα were less in TNF-α-treated cultures compared with controls ($P < 0.05$). Similarly, in the presence of LH or PGF2α, TNFα caused a reduction in DMα mRNA concentrations. Treatment with TNF-α in the presence of both LH and $PGF_{2\alpha}$ also caused slight, but non-significant, reductions in DMα mRNA. LH, $PGF_{2\alpha}$ and TNF-α were all without effect on concentrations of BoLA-DMβ mRNA in cultured luteal cells (Fig. 5).

Discussion

The present study is the first to demonstrate the presence of mRNA encoding Ii, DMα and DMβ in the CL of any species. These results are significant when considering the increase in the ability of luteal cells to stimulate T cell proliferation in response to $PGF_{2\alpha}$. Although we have previously demonstrated that reduction in progesterone synthesis is at least partly responsible for the increase in the ability of luteal cells to stimulate T lymphocyte proliferation following $PGF_{2\alpha}$ administration (Cannon *et al.* 2003), alteration in intracellular antigen processing could

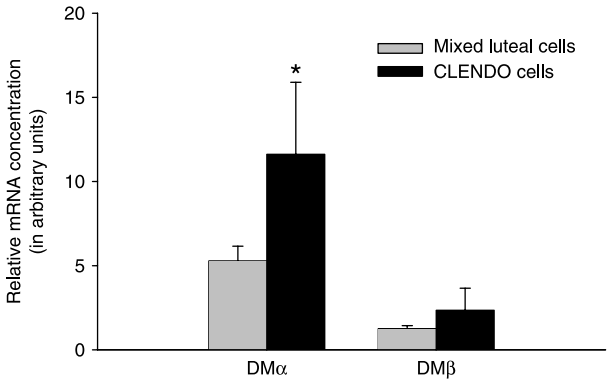

Figure 4 Steady-state concentrations of DMα and DMβ mRNA in cultures of mixed luteal cells and CLENDO cells. Bars represent concentrations of DMα and DMβ mRNA, as determined by RT-qPCR, in RNA extracted from cultures of mixed luteal cells (gray bars) or luteal endothelial cells (black bars) (mean±S.E.M., $n = 3$). *Significant difference between mixed luteal cell and luteal endothelial cell cultures.

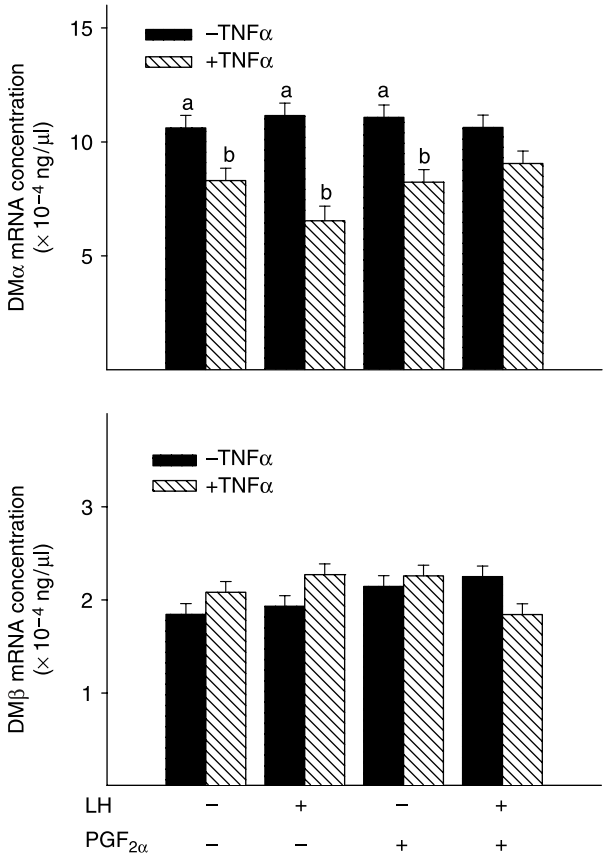

Figure 5 Steady-state concentrations of DMα mRNA (upper panel) and DMβ mRNA (lower panel) in cultures of mixed luteal cells. Bars represent concentrations of DMα and DMβ mRNA, as determined by RT-qPCR, in RNA extracted from luteal cells cultured in the presence or absence of LH and/or $PGF_{2\alpha}$, with (hatched bars) or without (black bars) TNF-α. Different letters denote significant differences between cultures in the presence or absence of TNF-α (mean±S.E.M., $n = 4$, $P < 0.05$).

also contribute to the enhanced ability of luteal cells from regressing CL to stimulate T cell proliferation. Alterations in DMα and DMβ expression near the end of the estrous cycle could catalyze a change in luteal cells that facilitates the process of luteal regression.

Steady-state concentrations of Ii mRNA were not different at any stage of the estrous cycle. The reason for this is not clear. Class II MHC expression on bovine luteal cells increases throughout the estrous cycle and following $PGF_{2\alpha}$ administration (Benyo *et al.* 1991), and since Ii is required for transport of class II MHC molecules to the cell surface, expression of Ii would be expected to parallel class II MHC expression. Given the observed lack of change in Ii mRNA during the estrous cycle or following administration of $PGF_{2\alpha}$, it appears that this gene is constitutively expressed in luteal tissue. It is possible that constitutive expression of Ii is sufficient to allow transport of class II MHC molecules to the cell surface. It must be noted, however, that the present study measured mRNA whereas the previous study measured cell surface proteins (Benyo *et al.* 1991). In that study, expression of class II MHC molecules by luteal cells was not measured beyond 12 h after a luteolytic dose of $PGF_{2\alpha}$. It is possible that class II MHC expression may increase dramatically by 24 h after $PGF_{2\alpha}$, similar to the increase in Ii mRNA concentrations observed in the present study.

Concentrations of DMα and DMβ mRNA were greatest in the CL during midcycle, and declined near the time of luteal regression. Since previous studies have shown that Ii and DM are coordinately regulated by the class II transactivating factor CIITA (Steimle *et al.* 1994, Chang & Flavell 1995), this result was unexpected in light of the observed lack of change in Ii mRNA concentration throughout the estrous cycle. The number of bovine luteal cells expressing class II MHC molecules, as well as the degree of class II MHC expression, both increase near the time of luteal regression (Benyo *et al.* 1991). The decline in DMα and DMβ mRNA concentrations in luteal tissue concurrent with increases in class II MHC expression may result in presentation of a repertoire of peptides to T cells during luteal regression that is different from that presented during midcycle. Administration of a luteolytic dose of $PGF_{2\alpha}$ to the cow did not induce changes in the steady-state concentrations of DMα or DMβ mRNA. The reason for the lack of effect of $PGF_{2\alpha}$ on DMα and DMβ expression is unknown, but it is evident that the control of expression of these genes in the CL is not regulated by $PGF_{2\alpha}$. Treatment of cultured luteal cells with TNF-α reduced concentrations of DMα, and elevation of TNF-α during luteal regression has been demonstrated (Ji *et al.* 1991, Shaw & Britt 1995). Elevation of TNF-α in the CL was not observed until after the decline in circulating and tissue concentrations of progesterone, but intra-luteal concentrations of TNF-α may increase prior to the decline in circulating concentrations of progesterone, which would explain the decline in DMα mRNA observed in late cycle CL. Steady-state concentrations of DMβ mRNA were not

affected by LH, TNF-α or $PGF_{2\alpha}$, indicating that multiple factors control the expression of DM in the CL.

The present study demonstrates the presence of Ii, DMα and DMβ mRNA in luteal tissue in amounts detectable using the relatively non-sensitive method of Northern analysis. That these messages are detectable by Northern analysis is suggestive of a source of mRNA other than infiltrating macrophages and B lymphocytes, which are relatively few in number compared with the total number of cells in the bovine CL. DMα and DMβ mRNA were also found in CLENDO cultures in the present study, which provides support for the hypothesis that a cell type in addition to macrophages in the CL expresses these components of the antigen-processing pathway. It is worth noting that the CLENDO cultures used in the present study are composed of very highly purified populations of luteal endothelial cells, since the concentration of CD31 mRNA in CLENDO cells was approximately tenfold greater than in mixed luteal cell cultures, whereas StAR mRNA is almost undetectable. Conversely, the mixed luteal cell cultures contain largely steroidogenic cells, but are likely to also contain small numbers of endothelial cells; large amounts of StAR mRNA were present in the mixed luteal cell cultures, whereas concentrations of CD31 mRNA were much less than those in CLENDO cultures. Steady-state concentrations of DMα were greater in CLENDO cell cultures compared with mixed luteal cell cultures, and although it was not significant, concentrations of DMβ mRNA also appeared to be greater in CLENDO cell cultures compared with mixed luteal cell cultures. However, DMα and DMβ mRNAs were also present in mixed luteal cell cultures in relatively high abundance. From the analysis of CD31 concentrations in the CLENDO and mixed luteal cell cultures, we have concluded that endothelial cells make up a very small proportion of the total cells in mixed luteal cell cultures. Therefore, it seems unlikely that endothelial cells account for the relatively large amount of DMα and DMβ mRNA present in mixed luteal cell cultures. We have not assessed the presence of macrophages in these cultures, but while it is unlikely that macrophages are present in the CLENDO cultures, due to the purification procedures used to derive these cultures, it is possible that significant numbers of macrophages are present in mixed luteal cell cultures. This would account for the presence of the relatively large amount of DMα and DMβ mRNA found in mixed luteal cell cultures. It is unfortunate that it was not possible to obtain a pure population of steroidogenic luteal cells for these studies, and it is not possible to conclude from this experiment whether or not steroidogenic cells express DMα and DMβ. However, these results clearly demonstrate that luteal endothelial cells express DMα and DMβ.

In conclusion, the present study demonstrates the expression of Ii, DMα and DMβ genes in luteal tissue. These genes encode intracellular antigen-processing proteins required for processing and presentation of antigenic peptides to T lymphocytes. The changes in DMα and DMβ expression observed near the end of the estrous cycle could result in presentation of an altered repertoire of self-peptides in the context of class II MHC molecules. This alteration of antigen presentation may have significant implications for regulation of luteal function via interaction of resident T lymphocytes with cells of the CL. However, since expression of the antigen-processing components examined in this study is greatest during mid-cycle, it is possible that presentation of antigen by class II MHC is necessary in the fully functional CL to maintain a state of immune tolerance to the tissue. Since immune tolerance would no longer be essential at the end of the estrous cycle, the decline in DMα and DMβ expression may serve to facilitate activation of immune cells that may participate in the process of regression. Future studies are needed to determine the role(s) played by the immune system in regulation of luteal function.

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References

Avva RR & Cresswell P 1994 *In vivo* and *in vitro* formation and dissociation of HLA-DR complexes with invariant chain-derived peptides. *Immunity* **1** 763–774.

Bagavandoss P, **Wiggins RC**, **Kunkel SC**, **Remick DG & Keyes PL** 1990 Tumor necrosis factor production and accumulation of inflammatory cells in the corpus luteum of pseudopregnancy and pregnancy in rabbits. *Biology of Reproduction* **42** 367–376.

Bakke O & Dobberstein B 1990 MHC class II-associated invariant chain contains a sorting signal for endosomal compartments. *Cell* **63** 707–716.

Benyo DF & Pate JL 1992 Tumor necrosis factor-α alters bovine luteal cell synthetic capacity and viability. *Endocrinology* **130** 854–860.

Benyo DF, **Haibel GK**, **Laufman HB & Pate JL** 1991 Expression of major histocompatibility complex antigens on the bovine corpus luteum during the estrous cycle, luteolysis, and early pregnancy. *Biology of Reproduction* **45** 229–234.

Bodmer H, **Viville S**, **Benoist C & Mathis D** 1994 Diversity of endogenous epitopes bound to MHC class II molecules limited by invariant chain. *Science* **263** 1284–1286.

Brännström M, **Giesecke L**, **Moore IC**, **van den Heuvel CJ & Robertson SA** 1994 Leukocyte subpopulations in the rat corpus luteum during pregnancy and pseudopregnancy. *Biology of Reproduction* **50** 1161–1167.

Cannon MJ & Pate JL 2003 Expression and regulation of interferon γ-inducible proteasomal subunits LMP7 and LMP10 in the bovine corpus luteum. *Biology of Reproduction* **68** 1447–1454.

Cannon MJ, **Petroff MG & Pate JL** 2003 Effects of prostaglandin $F_{2\alpha}$ and progesterone on the ability of bovine luteal cells to stimulate T lymphocyte proliferation. *Biology of Reproduction* **69** 695–700.

Castellino F, **Zhong G & Germain RN** 1997 Antigen presentation by MHC class II molecules: invariant chain function, protein trafficking, and the molecular basis of diverse determinant capture. *Human Immunology* **54** 159–169.

Cavicchio VA, **Pru JK**, **Davis BS**, **Davis JS**, **Rueda BS & Townson DH** 2002 Secretion of monocyte chemoattractant protein-1 by endothelial cells of the bovine corpus luteum: regulation by cytokines but not prostaglandin F2. *Endocrinology* **143** 3582–3589.

Chang CH & Flavell RA 1995 Class II transactivator regulates the expression of multiple genes involved in antigen presentation. *Journal of Experimental Medicine* **181** 765–767.

Del Vecchio RP & Sutherland WD 1997 Prostaglandin and progesterone production by bovine luteal cells incubated in the presence or absence of the accessory cells of the corpus luteum and treated with interleukin-1beta, indomethacin and luteinizing hormone. *Reproduction, Fertility and Development* **9** 651–658.

Denzin LK, **Hammond C & Cresswell P** 1996 HLA-DM interactions with intermediates in HLA-DR maturation and a role of HLA-DM in stabilizing empty HLA-DR molecules. *Journal of Experimental Medicine* **184** 2153–2165.

Fairchild DL & Pate JL 1991 Modulation of bovine luteal cell synthetic capacity by interferon-gamma. *Biology of Reproduction* **44** 357–363.

Fling SP, **Arp B & Pious D** 1994 HLA-DMA and -DMB genes are both required for MHC class II/peptide complex formation in antigen-presenting cells. *Nature* **368** 554–558.

Gorospe WC, **Hughes FM & Spangelo BL** 1992 Interleukin-6: effects on and production by rat granulosa cells in vitro. *Endocrinology* **130** 1750–1752.

Hartung S, **Rust W**, **Balvers M & Ivell R** 1995 Molecular cloning and *in vivo* expression of the bovine steroidogenic acute regulatory protein. *Biochemical and Biophysical Research Communications* **215** 646–653.

Ji I, **Slaughter RG**, **Ellis JA**, **Ji TH & Murdoch WJ** 1991 Analyses of ovine corpora lutea for tumor necrosis factor mRNA and bioactivity during prostaglandin-induced luteolysis. *Molecular and Cellular Endocrinology* **81** 77–80.

Jo T, **Tomiyama T**, **Ohashi K**, **Saji F**, **Tanizawa O**, **Ozaki M**, **Yamamoto R**, **Yamamoto T**, **Nishizawa Y & Terada N** 1995 Apoptosis of cultured mouse luteal cells induced by tumor necrosis factor-alpha and interferon-gamma. *Anatomy Record* **241** 70–76.

Katz JF, **Stebbins C**, **Appella E & Sant AJ** 1996 Invariant chain and DM edit self-peptide presentation by MHC II molecules. *Journal of Experimental Medicine* **184** 1747–1753.

Kenny N, **Herman JR**, **Barisas BG & Roess DA** 1991 Flow cytometric analysis of class I and II MHC antigens on ovine luteal cell types. In *Signaling Mechanisms and Gene Expression in the Ovary,*. Ed. G Gibori. New York: Springer-Verlag.

Khoury EL & Marshall LA 1990 Luteinization of human granulosa cells in vivo is associated with expression of MHC class II antigens. *Cell Tissue Research* **262** 217–224.

Komatsu K, **Manabe N**, **Kiso M**, **Shimabe M & Miyamoto H** 2003 Changes in localization of immune cells and cytokines in corpora lutea during luteolysis in murine ovaries. *Journal Experimental Zoology* **296A** 152–159.

Krusche CA, **Vloet TD**, **Herrler A**, **Black S & Beier HM** 2002 Functional and structural regression of the rabbit corpus luteum is associated with altered luteal immune cell phenotypes and cytokine expression patterns. *Histochemistry and Cell Biology* **118** 479–489.

Lawler DF, **Hopkins J & Watson DE** 1999 Immune cell populations in the equine corpus luteum throughout the oestrous cycle and early pregnancy: an immunohistochemical and flow cytometric study. *Journal of Reproductive Fertility* **117** 281–290.

Lightstone L, **Hargreaves R**, **Bobek G**, **Peterson M**, **Aichinger G**, **Lombardi G & Lechler R** 1997 In the absence of the invariant chain, HLA-DR molecules display a distinct array of peptides which is influenced by the presence or absence of HLA-DM. *PNAS* **94** 5772–5777.

Liptak AR, **Sullivan BT**, **Henkes LE**, **Wijayagunawardane MPB**, **Miyamoto A**, **Davis JS**, **Rueda BR & Townson DH** 2005 Cooperative expression of monocyte chemoattractant protein 1 within the bovine corpus luteum: evidence of immune cell-endothelial cell interactions in a coculture system. *Biology of Reproduction* **72** 1169–1176.

Lotteau V, **Teyton L**, **Péléraux A**, **Nilsson T**, **Karlsson L**, **Schmid SL**, **Quaranta V & Peterson PA** 1990 Intracellular transport of class II MHC molecules directed by invariant chain. *Nature* **348** 600–605.

Maric MA, **Taylor MD & Blum JS** 1994 Endosomal aspartic proteinases are required for invariant chain processing. *PNAS* **91** 2171–2175.

Marieke van Ham S, **Gruneberg V**, **Malcherek G**, **Broker I**, **Melms A & Trowsdale J** 1996 Human histocompatibilty leukocyte antigen (HLA)-DM edits peptides presented by HLA-DR according to their ligand binding motifs. *Journal of Experimental Medicine* **184** 2019–2024.

Morris P, **Shaman J**, **Attaya M**, **Amaya M**, **Goodman S**, **Bergman C**, **Monaco JJ & Mellins E** 1994 An essential role for HLA-DM in antigen presentation by class II major histocompatibility molecules. *Nature* **368** 551–554.

Neuvians TP, **Schams D**, **Berisha B & Pfaffl MW** 2004 Involvement of pro-inflammatory cytokines, mediators of inflammation, and basic fibroblast growth factor in prostaglandin $F_{2\alpha}$-induced luteolysis in bovine corpus luteum. *Biology of Reproduction* **70** 473–480.

Niimi M, **Nakai Y & Aida Y** 1995 Nucleotide sequences and the molecular evolution of the DMA and DMB genes of the bovine major histocompatibility complex. *Biochemical and Biophysical Research Communications* **217** 522–528.

Niimi M, **Nakai Y & Aida Y** 1996 Identification of bovine invariant chain (Ii) gene by nucleotide sequencing. *Biochemical and Biophysical Research Communications* **222** 7–12.

Nothnick WB & Pate JL 1990 Interleukin-1β is a potent stimulator of prostaglandin synthesis in bovine luteal cells. *Biology of Reproduction* **43** 898–903.

Pate JL & Condon WA 1982 Effects of serum and lipoproteins on steroidogenesis in cultured bovine luteal cells. *Molecular and Cellular Endocrinology* **28** 551–562.

Pate JL & Condon WA 1984 Effects of prostaglandin $F_{2\alpha}$ on agonist-induced progesterone production in cultured bovine luteal cells. *Biology of Reproduction* **31** 427–435.

Pate JL & Nephew KP 1988 Effects of in vivo and in vitro administration of prostaglandin $F_{2\alpha}$ on lipoprotein utilization in cultured bovine luteal cells. *Biology of Reproduction* **38** 568–576.

Penny LA, **Armstrong D**, **Bramley TA**, **Webb R**, **Collins RA & Watson ED** 1999 Immune cells and cytokine production in the bovine corpus luteum throughout the oestrous cycle and after induced luteolysis. *Journal of Reproductive Fertility* **115** 87–96.

Petroff M, **Coggeshall KM**, **Jones LS & Pate JL** 1997 Bovine luteal cells elicit major histocompatibility complex class II-dependent T-cell proliferation. *Biology of Reproduction* **57** 887–893.

Petroff MG, **Petroff BK & Pate JL** 1999 Expression of cytokine messenger ribonucleic acids in the bovine corpus luteum. *Endocrinology* **140** 1018–1021.

Petroff MG, **Petroff BK & Pate JL** 2001 Mechanisms of cytokine-induced death of cultured bovine luteal cells. *Reproduction* **121** 753–760.

Pru JK, **Lynch MP**, **Davis JS & Rueda BR** 2003 Signaling mechanisms in tumor necrosis factor alpha-induced death of microvascular endothelial cells of the corpus luteum. *Reproductive Biology and Endocrinology* **1** 17.

Riese RJ, **Wolf PR**, **Bromme D**, **Natkin LR**, **Villadangos JA**, **Ploegh HL & Chapman HA** 1996 Essential role for cathepsin S in MHC class II-associated invariant chain processing and peptide loading. *Immunity* **4** 357–366.

Roche PA & Cresswell P 1990 Invariant chain association with HLA-DR molecule inhibits immunogenic peptide binding. *Nature* **345** 615–618.

Sant AJ 1994 Endogenous antigen presentation by MHC class II molecules. *Immunology Research* **13** 235–267.

Shaw DW & Britt JH 1995 Concentrations of tumor necrosis factor alpha and progesterone within the bovine corpus luteum sampled by continuous-flow microdialysis during luteolysis in vivo. *Biology of Reproduction* **53** 847–854.

Steimle V, **Siegrist CA**, **Mottet A**, **Lisowska-Grospierr B & Mach B** 1994 Regulation of MHC class II expression by interferon-γ mediated by the transactivator gene CIITA. *Science* **265** 106–109.

Stewart RJ, **Kashour TS & Marsden PA** 1996 Vascular endothelial platelet endothelial adhesion molecule-1 (PECAM-1) expression is decreased by TNF-alpha and IFN-gamma. Evidence for cytokine-induced destabilization of messenger ribonucleic acid transcripts in bovine endothelial cells. *Journal of Immunology* **156** 1221–1228.

Townson DH & Pate JL 1994 Regulation of prostaglandin synthesis by interleukin-1 beta in cultured bovine luteal cells. *Biology of Reproduction* **51** 480–485.

Townson DH & Pate JL 1996 Mechanism of action of TNF-α-stimulated prostaglandin production in cultured bovine luteal cells. *Prostaglandins* **52** 361–373.

Townson DH, **O'Connor CL & Pru JK** 2002 Expression of monocyte chemoattractant protein-1 and distribution of immune cell populations in the bovine corpus luteum throughout the estrous cycle. *Biology of Reproduction* **66** 361–366.

Tsai S-J, **Wiltbank MC & Bodensteiner KJ** 1996 Distinct mechanisms regulate induction of messenger ribonucleic acid for prostaglandin (PG) G/H synthase-2, PGE (EP_3) receptor, and $PGF_{2\alpha}$ receptor in bovine preovulatory follicles. *Endocrinology;* **137** 3348–3355.

Vogt AB, **Arndt O**, **Hämmerling GJ & Kropshofer H** 1999 Quality control of MHC class II associated peptides by HLA-DM/H2-M. *Seminars in Immunology* **11** 391–403.

Young JE, **Friedman CI & Danforth DR** 1997 Interleukin-1β modulates prostaglandin and progesterone production by primate luteal cells in vitro. *Biology of Reproduction* **56** 663–667.

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