Relationship between different stages of the corpus luteum and the expression of the peroxisome proliferator-activated receptor \( \gamma \) protein in bovine large lutein cells

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Lutein cells produce progestins that support pregnancy. Steroidogenesis requires coordination of the anabolic and catabolic pathways of lipid metabolism. Peroxisome proliferator-activated receptors (PPAR) are transcription factors that are central in the regulation of lipid metabolism. Hence, they may play a role in regulation of the development and regression of the corpus luteum. The present study investigated the expression of PPAR-\( \gamma \) in during different stages of the corpus luteum. Lutein cells were isolated mechanically from non-pregnant and pregnant heifers on days 5, 12 and 20 of the oestrous cycle (\( n = 3 \) for each day). PPAR-\( \gamma \) in single cells was analysed by flow cytometry. PPAR-\( \gamma_1 \) and PPAR-\( \gamma_2 \) isoforms were distinguished by immunoblotting. The cell cycle of the lutein cells was measured by the flow cytometric quantification of DNA in single cells, using propidium iodide staining after ethanol fixation and RNAse treatment, and by the detection of the proliferating cell nuclear antigen (PCNA). The response of the cells to PPAR-\( \gamma \) agonist 15-deoxy-\( \Delta^{12,14} \) prostaglandin J\(_2\) (15dPGJ\(_2\), 200 and 490 nmol l\(^{-1}\)) with and without changing the cell cycle by the anti-apoptogenic drug aurintricarboxylic acid (ATA, 10 \( \mu \)mol l\(^{-1}\)) was used as an in vitro model to study the relationship between the cell cycle and PPAR-\( \gamma \). The concentration of PPAR-\( \gamma \) per cell from non-pregnant heifers was significantly higher on day 5 (3.40 ± 0.30 fmol) compared with that on day 12 (1.34 ± 0.18 fmol, \( P < 0.05 \)) and day 20 (0.55 ± 0.2 fmol, \( P < 0.05 \)). In pregnant heifers, the concentration of PPAR-\( \gamma \) was significantly (\( P < 0.01 \)) higher than in non-pregnant heifers. A decrease in the PPAR-\( \gamma_1 \) isoform relative to PPAR-\( \gamma_2 \) was observed in cells on day 12 of the oestrous cycle compared with day 5. The cell cycle (S phase portion in cells on days 5, 12 and 20: 16 ± 4%, 6 ± 4% and 4 ± 3%, respectively) and the portion of cells with PCNA correlated with the amount of PPAR-\( \gamma \) in non-pregnant heifers. ATA promoted the S phase in cells of non-pregnant heifers (day 12) and the endogenous agonist of PPAR-\( \gamma \) 15dPGJ\(_2\), inhibited the response to ATA in a dose-dependent manner, indicating that PPAR-\( \gamma \) plays a role in the arrest of the cell cycle in lutein cells to maintain their differentiated state.

Introduction

The corpus luteum develops from ovarian follicular mesenchymal thecal and epithelial granulosa cells to small and large lutein cells (Juengel et al., 1993; Monniaux et al., 1994). During differentiation, the luteinizing cells stop synthesizing oestrogen but maintain and increase the production of progestins (Selvaraj et al., 1994; Nicosia et al., 1995), chiefly 20a-dihydroprogesterone (Bowen et al., 1996). The differentiation of granulosa cells to large lutein cells is associated with a marked increase in metabolic functions which are characterized by increased activity of mitochondrial enzymes (Doody et al., 1990; Richards and Almond, 1994) to meet the energy requirements for the production of steroids. The increase in the expression of enzymes, required for the synthesis of progestins from cholesterol, is linked to increasing amounts of mRNA species encoding enzymes involved in cholesterol metabolism and to the expression of novel mitochondrial enzymes (Doody et al., 1990). The alteration of the expression of steroidogenic enzymes is regulated by hormones and other factors, such as insulin, insulin-like growth factor I, LH, transforming growth factor \( \beta \) and their receptors (Monniaux et al., 1994). The differentiation is also accompanied by the expression of receptors involved in the uptake of lipids (Funkenstein et al., 1984). They provide substrates for the steroid synthesis in granulosa and luteinizing cells and the energy required for steroid synthesis from the \( \beta \)-oxidation of fatty
acids (Funkenstein et al., 1984; Amsterdam and Rotmensch, 1987).

The mechanisms underlying the alteration of gene expression in luteinizing granulosa cells are not completely understood. It has been reported (Pall et al., 1997; Sterneck et al., 1997; Löhre et al., 1998) that transcription factors are involved, which mediate the transduction of hormonal signals, including those arising from lipids, to targets at the DNA. A group of transcription factors, the peroxisome proliferator-activated receptors (PPARα and γ), are thought to be key regulators in cellular lipid homeostasis (Kliewer et al., 1997) which requires co-ordination between peroxisomes and mitochondria during steroidogenesis (Mendis-Handagama et al., 1995). PPAR-γ regulates the expression of genes encoding enzymes of the cholesterol synthesis pathway, such as 3-hydroxy-3-methylglutaryl-CoA synthase (Rodriguez et al., 1994) and lipoprotein lipase (Schoonjans et al., 1996) which mediates uptake of fatty acids and cholesterol.

In contrast to PPAR-γ, the expression of PPAR-α is regulated by glucocorticoids (Lemberger et al., 1994). The transcription factor C/EBPα, a member of the CCAAT enhancer binding protein (C/EBP) family, stimulates the expression of PPAR-γ (Wu et al., 1995). In turn, the C/EBPα gene is regulated by gonadotrophins and is the only known transcription factor that is induced promptly in response to an ovulatory dose of LH and to the LH surge naturally occurring during ovulation in many species (Pall et al., 1997; Sterneck et al., 1997). C/EBPα stimulates not only the expression of PPAR-γ but also, in co-operation with C/EBPγ of cyclooxygenase 2, also called prostaglandin endoperoxide synthase 2 (Sirois and Richards, 1993), which is important in the production of prostaglandins from arachidonic acid metabolism (Reginato et al., 1998).

PPAR-γ is activated by binding arachidonic acid and type D3 prostaglandins (Kliewer et al., 1997) of which 15-deoxy-Δ12,14 PGJ2 (15dPGJ2) is the most potent (Forman et al., 1995), whereas PGE2 (PGE2) is the most potent (Forman et al., 1995) which requires co-ordination between lipid and transcription factors in the cellular metabolic compartments that provide lutein cells with substrates for steroidogenesis. Enhanced steroidogenesis is accompanied by a loss of lutein cell cycle activity (Fields et al., 1992). The steroidogenesis ceases and the corpus luteum regresses when fertilization does not take place. The corresponding regulatory events are poorly understood. It is hypothesized that the expression rate of PPAR-γ is linked to the developmental stages of the corpus luteum. Hence, in the present study, the expression of PPAR-γ protein in relation to the cell cycle activity during different stages of the corpus luteum was investigated.

Materials and Methods

Materials

PPAR-γ antibodies to the amino acid sequence 284–298 of the PPAR-γ protein were purchased from Cayman-Alexis (Grüneberg). Standards beads for the calibration of fluorescence intensities were obtained from FCSC (Leiden). All other chemicals, hormones and antibodies were from Sigma (Deisenhofen), unless stated otherwise.

Animals

Dairy heifers at 18–20 months old and weighing between 430 and 470 kg were used in the experiments. Heifers with a regular oestrous cycle (day 5, day 12, day 20, oestrus = day 0) were used. Detection of oestrus was performed twice a day. In addition, rectal palpation of the ovaries and ultrasonic diagnosis were conducted to gain more information on the stage of the oestrous cycle. Pregnant heifers (day 0 = oestrus + artificial insemination) were killed on days 5, 12 and 20 (three animals on each day).

Lutein cell preparation

Corpora lutea were obtained by ovariectomy performed on days 5, 12 and 20 (three animals on each day) of the
was checked by flow cytometry; the viability was > 95%.

40 µmol propidium iodide l–1. Uptake of propidium iodide
by gating the cells in the flow cytometric histogram. Cell
analysis, the percentage of large lutein cells increased > 95%
small lutein cells separated by flow cytometry were 60 ± 10

Cell cycle analysis by flow cytometry

Freshly prepared and cultured cells (days 5, 12 and 20 of
the oestrous cycle) were used. The cultured cells were
incubated for 24 h at 37°C in culture medium with the
solvent and with the effectors 15dPGJ2 and ATA. A stock
solution of 15dPGJ2 dissolved in methylacetate was diluted
with 5 mmol sodium phosphate 1–1 (pH 7.1) and ATA was
dissolved in 50 mmol NaHCO3 1–1. The solutions were added
(1 volume to 20 volumes of the culture medium) in final
concentrations of 200 and 490 mmol 15dPGJ2 1–1 (0.01% (v/v)
methylacetate) and 10 µmol ATA 1–1. Freshly prepared
and cultured cells were fixed in ethanol (70%), washed and
treated with RNase solution (100 µl l–1 in PBS, 37°C,

Detection of proliferating cell nuclear antigen

Cells were fixed in ethanol (70%, v/v), washed and treated
with RNAase (1 mg ml–1 in PBS, 37°C, 30 min) as described
above. The cells were incubated with purified monoclonal
IgG1 mouse antibody against PCNA (final concentration
5 µg ml–1) in the dark for 1 h at room temperature. The
antibody was conjugated with fluorescein isothiocyanate
isomer (FITC). A negative control was obtained by
incubating the cells with a non-specific mouse IgG2a-FITC
conjugate (final concentration 5 mg l–1; both antibodies from
DAKO, Hamburg). After incubation, the cells were washed
by centrifugation at 350 g for 5 min, re-suspended with DNA
staining medium (propidium iodide, 70 µmol l–1, in HBS)
and incubated in the dark for 30 min at room temperature.
After washing and centrifugation at 350 g for 5 min, the cells
were resuspended in HBS and the dual fluorescence was
analysed by flow cytometry.

PPAR-γ immunofluorescence

Lutein cells were fixed in methanol (–20°C) for 4 min,
using one volume of cell suspension and ten volumes of
methanol. The cells were centrifuged (150 g, 4°C, 5 min),
re-suspended in PBS containing 0.2% (w/v) BSA (pH 7.4)
and aliquots were plated (24-well plastic microplates). The
cells were incubated (1 h, 22°C) with rabbit-anti-PPAR-γ
antibodies used in a final concentration of 2.5 µg ml–1. The
unbound antibodies were washed out and the cells were
incubated for 1 h at 22°C with phycoerythrine-conjugated
anti-rabbit F(ab’)2 antibody fragments (PBS, pH 7.2, 0.2%
(w/v) BSA) or with FITC-conjugated anti-rabbit-F(ab’)2
antibody fragments (PBS, pH 7.4, 0.2% (w/v) BSA). After
washing by centrifugation at 150 g, 4°C, 5 min and re-
suspension in PBS (pH 7.4, 0.2% (w/v) BSA), the cells were
analysed by flow cytometry. A control of the non-specific

Flow cytometry

Quantitative analysis of cellular fluorescence was
performed by flow cytometry to analyse the cells
simultaneously according to size, granularity, LH receptors
and PPAR-γ expression (proportion of cells expressing
PPAR-γ and PPAR-γ content per cell). The analysis of LH
receptors was carried out according to the method of Fields
et al. (1992) and the flow cytometric analysis was conducted
as described by Lührke et al. (1997). Briefly, an argon laser-
equipped flow cytometer (Coulter-Elite, Krefeld) recording
emissions of multiple fluorescence (green, orange, red)
excited at 488 nm (counting 5000 cells) was used. Cells of
interest were identified by three successive steps: (i) by
establishing a histogram on the basis of cell size and
granularity; (ii) by establishing the fluorescence histogram;
and (iii) by projecting the fluorescence into the
size–granularity histogram. The cells were gated and the
proportion of cells and their fluorescence intensity arising
from a second fluorogenic label were automatically
calculated.
fluorescence was obtained by omitting the PPAR-γ-specific antibody. The corresponding value was automatically subtracted by the flow cytometer and the percentage of fluorescence-positive cells and the fluorescence intensity per cell were recorded. Quantification of the concentration of PPAR-γ was carried out as described by Löhrke et al. (1998). Briefly, a calibration of the flow cytometric fluorescence intensity by standards beads (p26) was conducted and the fluorescence intensity of the cells was converted into concentrations with the aid of the calibration curve.

**Immunoblotting of PPAR-γ, and PPAR-γ, isoforms**

Cells (days 5, 12 and 20 of the oestrous cycle and days 5 and 12 after PGF2α) were lysed by cold aqueous 0.1% (w/v) sodium citrate and 0.1% (v/v) Triton-X100, and the lysate was fractionated by centrifugation (24000 g, 1 h, 4°C). The supernatant of the ultracentrifugate was used immediately dissolved in the blot buffer containing 2.5% (v/v) sodium citrate and 0.1% (v/v) Triton-X100, and the lysate was fractionated by centrifugation (240 000 g, 1 h, 4°C). The nitrocellulose was washed and incubated with PPAR-γ antibodies dissolved in the blot buffer containing 2.5% (v/v) horse serum and used in a final concentration of 1 µg ml–1. The nitrocellulose was blocked by horse serum (5%, v/v) and PPAR-γ was detected by rabbit anti-PPAR-γ antibodies dissolved in the blot buffer containing 2.5% (v/v) horse serum and used in a final concentration of 1 µg ml–1. The nitrocellulose was washed and incubated with peroxidase-conjugated anti-rabbit-F(ab’)2 according to the manufacturer’s instructions (Sigma). The stain was developed by diaminobenzidine (0.3%, w/v) and H2O2 (0.01%, v/v) in 100 mmol Tris l–1, pH 7.2, as described by Löhrke et al. (1993, 1998). The bands were analysed by scanning the blots and densitometry (Herolab program package, Wiesloch).

**Statistical analysis**

Unless otherwise stated, experimental data are presented as the mean ± SD of duplicate measurements of three independent experiments. ANOVA and Tukey’s comparison methods were used for comparisons among multiple means. Assessment of the significance of differences in relative data was accomplished by a paired t test. Percentage data obtained by flow cytometry were analysed by chi-squared analysis (internal program package of the flow cytometer).

**Results**

**Corpus luteum morphology in relation to the lutein cell cycle**

The diameter of the corpus luteum increased from early dioestrus (day 5) to mid-dioestrus (day 12) and decreased with increasing age towards pro-oestrus (day 20) (Fig. 1a–c, insets). The results of the cell cycle analysis indicated the highest proportion of cells in S phase occurred on day 5 (16 ± 4%, n = 3). The proportion of cells in S phase decreased on day 12 (6 ± 4%, n = 3) and the lowest proportion was observed on day 20 (3 ± 3%, n = 3) (Fig. 1a–c).

The agonist of PPAR-γ, 15dPGJ2, used at concentrations of 200 and 490 nmol l–1 did not affect the proportion of cells in S phase after incubation of lutein cells on day 12 of the oestrous cycle (Fig. 2). ATA (10 µmol l–1) significantly increased the proportion of cells in S phase (18 ± 1%, P < 0.05, n = 3) after incubation with the drug for 24 h. Activation of PPAR-γ by 15dPGJ2 inhibited the stimulatory effect of ATA on the percentage of cells in S phase (Fig. 2).

**Relationship between PCNA and cellular DNA content**

The expression of PCNA protein was monitored in different phases of the cell cycle by dual fluorescence analysis involving immunostaining of the PCNA protein and the DNA stain arising from propidium iodide (Fig. 3). Measurement of the DNA content per cell indicated an increase in the proportion of cells in the G0/G1 phase with increasing age of the corpus luteum (Fig. 3). In this analysis, the control data from days 5 and 12 (three animals in each case) were pooled as they did not differ (Fig. 3). Quantitative results of the flow cytometric histograms (Fig. 3) are

**Table 1. Relationship between DNA content and proliferating cell nuclear antigen (PCNA) in lutein cells from days 5 and 12 of the oestrous cycle in non-pregnant heifers**

<table>
<thead>
<tr>
<th>Quadrant</th>
<th>Phase of the cell cycle</th>
<th>Percentage (± SD)</th>
<th>Phase of the cell cycle</th>
<th>PCNA (+/−)</th>
<th>Percentage (± SD)</th>
<th>Phase of the cell cycle</th>
<th>PCNA (+/−)</th>
<th>Percentage (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>G + S + M</td>
<td>18 ± 3</td>
<td>I</td>
<td>G + S + M</td>
<td>+</td>
<td>27 ± 5</td>
<td>I</td>
<td>G + S</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>II</td>
<td>III</td>
<td>G + S + M</td>
<td>−</td>
<td>11 ± 5</td>
<td>II</td>
<td>M</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>IV</td>
<td>III</td>
<td>G + S + M</td>
<td>−</td>
<td>55 ± 4</td>
<td>III</td>
<td>G + S</td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>IV</td>
<td>IV</td>
<td>G + S + M</td>
<td>−</td>
<td>7 ± 3</td>
<td>IV</td>
<td>M</td>
</tr>
<tr>
<td>I + II</td>
<td>G + S + M</td>
<td>18 ± 3</td>
<td>I + II</td>
<td>G + S + M</td>
<td>+</td>
<td>38 ± 4</td>
<td>I + II</td>
<td>G + S + M</td>
</tr>
</tbody>
</table>

Cells, symbols and data are identical to those described in Fig. 3. In addition, + and – stand for PCNA-positive and -negative cells.

(a) Represents non-specific immunofluorescence (control), (b) and (c) represent data from days 5 and 12 of the oestrous cycle, respectively (n = 3 for each day).

Quadrant I indicates the proportion of cells in G0/G1 phase (G) and S phase (S). Quadrants II, III, IV represent the proportion of the cells showing the phases G2/M (M), G and M, with and without expression of PCNA protein, as illustrated in Fig. 3.

1. The differences (b) I – (a) I and (c) I – (a) I yield the PCNA-positive fraction (%).

2. Only the difference (b) I + II – (a) I + II was significant (20 ± 3%, P < 0.05).
presented (Table 1). The proportions of PCNA-positive cells in the G0/G1 phase relative to the total number of cells in G0/G1 were 30% and 7% in cells from days 5 and 12, respectively, indicating activated G1-lutein cells occurred predominantly in the cell pool from day 5. In the analysis of PCNA, non-specific mouse IgG2a-FITC was used as a negative control (Fig. 3a (quadrant I + II) and Table 1; 18 ± 3%, n = 4, two non-pregnant animals per group). In non-pregnant heifers, the proportion of PCNA-positive cells on day 5 was higher (38 ± 4%, n = 3) than the proportion observed on day 12 (Fig. 3b,c (quadrant I+II), and Table 1; 20 ± 3%, n = 3). An ectopic expression of PCNA in lutein cells on day 12 was not detected (Table 1) and the expression in cells from non-pregnant heifers on day 20 was not different from the control (data not shown).

Comparison of PPAR-γ protein expression in lutein cells between non-pregnant and pregnant heifers

Flow cytometrical quantification of the PPAR-γ protein in day 5, day 12 and day 20 non-pregnant and pregnant heifers is shown (Fig. 4). A decrease in the proportion of PPAR-γ-positive cells was detected in large lutein cells from non-pregnant heifers on day 5 > day 12 > day 20 (Fig. 4a). In contrast, the portion of PPAR-γ-positive cells in pregnant heifers only differed significantly between day 5 and day 20 (Fig. 4a). The number per cell was significantly (P < 0.05, n = 3) higher in pregnant heifers compared with non-pregnant heifers (Fig. 4b). There were marked differences in the cellular PPAR-γ concentration among the
different stages of the corpus luteum (Fig. 4b). The cellular PPAR-γ content of day 5 and day 20 lutein cells decreased from 4.5 ± 0.5 to 1.0 ± 0.8 fmol per cell in pregnant heifers (Fig. 4b). In non-pregnant heifers, the corresponding values were 3.4 ± 0.3 and 0.5 ± 0.2 fmol per cell.

Detection of PPAR-γ isoforms

Lutein cells express PPAR-γ1 with a molecular mass of 52 kDa and PPAR-γ2 with a molecular mass of 56 kDa (Fig. 5). The results of the analysis of the changes in the molecular pattern of PPAR-γ are also presented (Fig. 5). The different stages of the corpus luteum are distinguished by an alteration in the ratio of PPAR-γ1:PPAR-γ2. A decrease in the PPAR-γ1 isoform is detectable in day 12 large lutein cells (50% of the value on day 5, P < 0.05) of non-pregnant animals (Fig. 5).

Information about the physiological background of the reduction in the PPAR-γ proteins was obtained by treating...
non-pregnant animals on day 4 + 12 h with the PPAR-γ antagonist PGF2a, as at this stage the cells of the corpus luteum apparently express the complete set of PPAR-γ proteins. The PPAR-γ1 protein was markedly reduced (40% of the non-treated control value, P < 0.02), whereas the PPAR-γ2 isoform decreased less markedly (Fig. 5).

**Discussion**

PPAR-γ is expressed in numerous tissues albeit at different concentrations (Braisant et al., 1996). Nunez et al. (1997) reported that mRNA from PPAR is present in oestrogen-dependent reproductive tissues and PPARs can activate oestrogen-dependent genes. However, this report only provided evidence for the expression of PPAR-α mRNA in granulosa and theca cells of the ovary and in the corpus luteum. Investigations on PPAR-γ expression in female reproductive cells are lacking, except for the study of Löhrke et al. (1998) in which expression of PPAR-γ mRNA and protein was reported in bovine lutein cells. The results also showed that the PPAR-γ proteins are active and are involved in the control of progesterone production, a functional differentiation marker of lutein cells (Murdoch, 1995; Juengel et al., 1995).

The results of the present study show that the concentration of PPAR-γ proteins differs among different luteal stages. The highest expression of PPAR-γ was observed in the cells at early dioestrous (day 5). This expression decreased during mid-dioestrous (day 12) and during pro-oestrous (day 20). The expression of PPAR-γ was significantly higher in the cells from pregnant heifers, compared with non-pregnant heifers. Information about the pathways mediating the temporal course of the expression of PPAR-γ proteins was obtained by treating animals at early dioestrous with an antagonist to PPAR-γ, PGF2α (Reginato et al., 1998). The lutein cells responded with a decrease in the PPAR-γ proteins, especially PPAR-γ1, indicating PPAR-γ is a target for the action of the prostaglandin, which is reported to have strong luteolytic activity in many mammalian species (Tsai and Wiltbank, 1998). These results indicate PPAR-γ has a role in the maintenance of a differentiated state in large lutein cells. In other mammalian cells, both PPAR-γ isoforms are negatively regulated by tumour necrosis factor α (TNF-α) (Zhang et al., 1996), a cytokine that may be involved in luteolysis (Gaytan et al., 1998). However, low constitutive expression of PPAR-γ was sufficient to block partially the inhibitory effects of TNF-α on the expression of genes regulated by PPAR-γ (Zhang et al., 1996). The ratio of the two isoforms of PPAR-γ varies both among species and tissues (Vidal-Puig et al., 1997; Ma et al., 1998). PPAR-γ1 has been found in rodents as a minor component in adipose and non-adipose tissues, whereas PPAR-γ1 mRNA in bovine spleen, lung and ovary was expressed at a higher level than PPAR-γ2 mRNA (Sundvold et al., 1997). Ma et al. (1998) suggested that the function of the PPAR-γ1 and PPAR-γ2 isoforms in avian oestrogen-responsive uropygial gland was different: PPAR-γ1 may be involved in peroxisome proliferation, whereas PPAR-γ2 may be involved in the regulation of lipid metabolism. In contrast, to date, nothing is known about the role of the PPAR-γ isoforms in lutein cell differentiation. Regulation of lutein cell differentiation should involve control of the cell cycle. Studies in domestic ruminants indicate that small (theca-derived) lutein cells proliferate, whereas large (granulosa-derived) lutein cells do not (Sawyer, 1995). The proliferative activity in bovine lutein steroidogenic cells in different stages of the corpus luteum is incompletely understood (Niswender et al., 1994). The results of the present study demonstrate that the proportion of large lutein cells in the S phase of the cell cycle that express PCNA decreased during mid-dioestrous (day 12) compared with early dioestrous (day 5). This observation corresponds to the morphological changes in the corpus luteum and with the concentration of PPAR-γ per lutein cell. PPAR-γ exerts an inhibitory effect on transcription factors, such as those of the signal-transducing activators of transcription (STAT) family (Ricote et al., 1998), and binds cofactors, including members of the src family (Shao et al., 1998) that are involved in regulation of the cell cycle. However, the mode of action of PPAR-γ on the cell cycle in lutein cells remains to be elucidated. A prerequisite for PPAR-γ activities on the transcription of target genes is activation by an agonist, including long chain fatty acids and type PGD2 prostanooids (Forman et al., 1995; Kliwer et al., 1995). The most potent PGD2 derivative, 15dPGJ2, is produced by macrophages that also express PPAR-γ (Ricote et al., 1998) and are present in the corpus luteum (Gaytan et al., 1998; Penny et al., 1998; Zhao et al., 1998). The number of macrophages identified by the surface antigen CD68 is parallel to the functional activity of the human corpus luteum, indicating bi-directional communication between macrophages and steroidogenic cells or regulation of both cell populations of the corpus luteum by a similar mechanism (Gaytan et al., 1998). The results of the present study indicate that the corresponding signal transduction may be mediated by PPAR-γ. The activity of macrophages may change with changes in the concentrations of activating and suppressing factors, such as interferon γ and progesterone. The steroid is known to act as a potent antagonist of some activities of macrophages, including superoxide production (Sugino et al., 1996). Hence, a decrease in ovarian production of PPAR-γ agonists appears to reduce the ectopic expression of PPAR-γ in a time-dependent manner. However, the action of macrophages on lutein cells, via the potent PPAR-γ agonist 15dPGJ2, is unknown, whereas the luteotrophic effects of type D prostaglandins are established in vitro and in vivo (Zelinski-Wooten and Stouffer, 1990).

The present investigation of the effect of 15dPGJ2, focused on lutein cells at mid-dioestrous since they are thought to contribute most of the circulating progestins, whereas oestrogens disappear from circulation (Park-Sarge et al., 1995). These cells almost ceased their proliferative activity as demonstrated in the present study and by Fields et al. (1992). ATA is a drug that stimulates progress of the cell cycle in lutein cells at mid-dioestrous (Löhrke et al., 1998). In addition, ATA exerts anti-apoptogenic effects on several types of cell (Catchpoole and Stewart, 1994; Posner et al., 1995) including lutein cells (Löhrke et al., 1998; Viergutz et al., 1999). Hence, the effect of 15dPGJ2 on cell cycle activity was investigated using the ATA model. The results showed that the PPAR-γ agonist
suppresses an ATA-induced increase in the percentage of cells in S phase in a dose-dependent manner. These results indicate the reversibility of the cell cycle arrest in mid-dioestrus cells and a role for PPAR-γ in arresting the cell cycle to maintain the differentiated function of lutein cells.

In summary, the results of the present study demonstrate a downregulation of the expression PPAR-γ and PCNA proteins in large lutein cells of the ageing corpus luteum, indicating a role in the maintenance of differentiated luteal cells. This proposal is supported by the experiments showing that the near arrest of cell cycle activity in lutein cells at mid-dioestrus can be stimulated by ATA and the natural PPAR-γ agonist, 15dPGJ₂, reverses the response. In addition, the naturally occurring decrease in the percentage of cells expressing PPAR-γ and in the concentration of PPAR-γ per cell can be simulated by exposing lutein cells from early dioestrus to PGF₂α, which acts luteolytically on the mid-dioestrus corpus luteum and inactivates PPAR-γ. The cells respond to the prostanoid by downregulating PPAR-γ, predominantly by decreasing the PPAR-γ protein, indicating that this isoform is a target for the luteolytic action of PGF₂α.

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