The arrays were hybridized for 16 hours at 45°C. After being hybridized, the arrays were washed using the fluidics station (Affymetrix, Santa Clara, CA) and scanned on a GeneChip Scanner 3000 (Affymetrix, Santa Clara, CA). Expression analysis of all experiments was performed with GeneSpring 5.1 (Silicon Genetics, Redwood City, CA) using analysis data generated by Microarray Suite software. Differential expression was defined as those transcripts that had a difference of twofold or more. Performed with GeneSpring software, the cross-gene-error model was used in combination with a one-way analysis of variance (ANOVA) (significance level set at $p < 0.05$).

Relative RT-PCR Analysis

Total RNA from frozen mouse CL was isolated using Tri–Reagent following the manufacturer's instructions. One microgram of total RNA was reverse-transcribed at 42°C with the Advantage RT–for-PCR kit (Promega, Madison, WI) and later diluted to a final volume of 100 µl. The PCR reaction mixture, containing specific oligonucleotide primers (500 pmol), deoxyribonucleotide triphosphate (150 µM), and *Ex Taq* DNA polymerase (1 Unit) (Takara, Kyoto, Japan), was added to each tube containing 5 µl of reverse transcription product. Each PCR reaction also included β-actin primers used as internal controls. Before proceeding with the semi-quantitative PCR, conditions were established such that the amplification of the products was in the exponential phase, and the assay was linear with respect to the amount of input cDNA. Band intensity was determined by using ImageJ software from NIH. Each experiment was repeated three times. A student *t* test was used to determine whether differences between groups were statistically different.

Western Blotting Analysis

Corpora lutea from wild-type mice on days 15 and 19 of pregnancy and from $PGF_{2\alpha}$ receptor knockout mice were homogenized in ice–cold lysis buffer (10 mM Tris–Cl, pH 8.0; 150 mM NaCl, 1% Nonidet p–40, 0.5% sodium deoxycholate, 0.1% SDS, 40 µM PMSF, 0.3 µM aprotinin, and 1 µM leupeptin). This was followed by 30-min incubation on ice and centrifugation at 10,000-x g for 20 min at 4°C. The

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supernatant was transferred to new tubes, aliquoted and stored at -70°C until the time of electrophoresis. An aliquot of the supernatant was kept for protein measurement, using bovine serum albumin as a standard. Samples were denatured by adding a sample buffer (62.5 mM Tris-HCL, pH6.8, 2% SDS, 10% glycerol, 0.01% bromophenol blue), followed by boiling for 10 minutes. Thirty micrograms of protein were separated on 10% SDS–PAGE gels in Tris-glicine, 0.1% SDS buffer, and transferred to nitrocellulose paper in 25mM Tris, 192 mM glycine, and 20% methanol buffer at 250 mA for 1.5 h. Blots were incubated for 2 hours at RT in 5% non-fat dry milk to block unspecific binding. Blots were then washed and incubated overnight at 4 °C with anti-P450 aromatase antibody (Serotech, UK) at a 1/4000 dilution, with anti-superoxide dismutase 2 antibody (Calbiochem) at a 1/800 dilution or with anti-β-actin antibody (Santa Crus Biotechnology) at a 1/1000 dilution. After three washes, blots were incubated with a corresponding secondary antibody conjugated to horseradish peroxidase (1/20000 dilution) for 2 hours at room temperature. Protein-antibody complexes were visualized using Western Blotting Luminol Reagent, following the manufacturer's protocol (Santa Cruz Biotechnology).

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Results

Global Analysis of Gene Expression in Mouse Corpus Luteum on Day 19 of Pregnancy

Probes derived from total RNA extracted from CL of wild-type or $PGF_{2\alpha}$ receptor knockout mice were hybridized to the mouse 430 2.0 chip array from Affymetrix. A total of four pairs of samples and comparisons was performed. Figure 1 shows a representative distribution of the intensity of all genes present in the microarray. The average Pearson correlation coefficient was 0.929, indicating similar levels of expression between samples. A total of 1,373 genes demonstrated at least a two-fold change in expression. The statistical significance of these changes was determined by ANOVA included in the GeneSpring software. Only genes with a net intensity equal to or higher than 10 times the background were chosen for further analysis. Between these highly expressed genes, 169 were down-regulated and 101 were up-regulated in the CL of $PGF_{2\alpha}$ receptor knockout mice. Of these genes, eleven are involved in steroid synthesis and ROS metabolism. To provide a more complete analysis, we report the expression of those genes that were significantly affected by the lack of $PGF_{2\alpha}$ receptor expression as well as the expression of several key genes involved in luteal steroidogenesis and ROS metabolism.

Expression Levels of Steroidogenic Genes on Day 19 of Pregnancy in Corpus Luteum of Wild-type or $PGF_{2\alpha}$ Receptor Knockout Mice

To facilitate the interpretation and integration of the results, a simplified scheme of rat luteal steroidogenesis is presented in figure 2A. As shown in figure 2B (top panel), no changes in mRNA levels of the scavenger receptor, class B, type 1 (*Scarb1*), the Steroidogenic Acute Regulatory (*Star*), and the Benzodiazepine Receptor peripheral-type (*Bzrp*) were found between knockout and wild-type animals. Analysis of the relative expression levels of these genes shows high relative expression of *Scarb1* and *Star*, both in wild-type and $PGF_{2\alpha}$ receptor knockout mice (Figure 2A, lower panel). Messenger RNA relative expression of *Bzrp* was 10 times lower than *Star* relative expression.

A significant increase (3.5-fold) in mRNA levels of the enzyme cholesterol 25-hydroxylase (*Ch25h*) was found in wild-type animals when compared to $PGF_{2\alpha}$ receptor knockout mice. No differences were found between wild-type and $PGF_{2\alpha}$ receptor knockout mice in the expression of cytochrome P450scc (*Cyp11a1*) and 3β-hydroxysteroid dehydrogenase, Δ^5 isomerase (*Hsd3b*) type 1 and 7 (Figure 2B, top panel). *Cyp11a1* and *Hsd3b1* were found to be the most highly expressed genes, not only between the steroidogenic genes (Figure 2B, lower panel), but also among all the genes present in the microarray used (not shown). No expression of other *Hsd3b* mRNA isoforms was found. As previously reported a significant increase in 20α-hydroxysteroid dehydrogenase (*Akr1c18*) mRNA levels was found in wild-type animals (Figure 2B).

Significant differences in $P450_{17\alpha}$-hydroxylase/C17-20 lyase (*Cyp17a1*) and cytochrome P450 aromatase (*Cyp19a1*) mRNA levels were found between $PGF_{2\alpha}$ receptor knockout mice and wild-type animals (Figure 2B, top panel). High mRNA levels of *Cyp19a1* were found in $PGF_{2\alpha}$ receptor knockout mice, whereas *Cyp17a1* mRNA levels were higher in wild-type mice.

The chip array used contains probes for all known 17β-hydroxysteroid dehydrogenase (*Hsd17b*) isoforms; however, only *Hsd17b* types 1, 4 and 7 mRNA were found to be expressed in the corpus luteum. *Hsd17b1* and *Hsd17b4* relative expression was low and no differences were observed between wild-type and $PGF_{2\alpha}$ receptor knockout mice (Figure 2B, lower panel). Interestingly, *Hsd17b7* mRNA levels were significantly higher in $PGF_{2\alpha}$ receptor knockout mice when compared to wild-type animals (Figure 2B, top panel).

Luteal Antioxidant Capability in Corpus Luteum of Wild-Type or $PGF_{2\alpha}$ Receptor Knockout Mice on Day 19 of Pregnancy

A simplified scheme of oxygen radical metabolism in presented in Figure 3A. As shown in figure 3B, it was found that the mouse corpus luteum expresses messenger RNA for all superoxide dismutase enzymes (SOD) i.e. *Sod1*, *Sod2*, and *Sod3* (soluble or copper-zinc, mitochondrial or manganese, and

extracellular, respectively). *Sod2* was found to be the most highly expressed (Figure 3B, lower panel), and its mRNA levels were significantly lower in wild-type animals when compared to levels found in $PGF_{2\alpha}$ receptor knockout mice (Figure 3B, top panel). Similarly, a decrease of 4.4-fold in *Sod3* mRNA levels in wild-type animals was observed when compared to knockout animals, whereas mRNA levels of *Sod1* were no different between these groups. Interestingly, mRNA levels of a protein called copper chaperone for SOD1 (*Ccs*) were significantly lower in wild-type animals.

The results also showed that the mRNA of all members of the peroxiredoxin (*Prdx*) family of proteins are expressed in the CL. *Prdx1*, *Prdx2*, *Prdx3*, and *Prdx6* mRNA were found to be highly expressed, while *Prdx4* and *Prdx5* were found to be present at low levels (Figure 3B, lower panel). Low relative expression levels of catalase (*Cat*) were found (Figure 3, lower panel). Messenger RNA levels of *Prdx6* were significantly lower in wild-type mice when compared to $PGF_{2\alpha}$ receptor knockout animals (Figure 3B, top panel).

High luteal mRNA levels for glutathione peroxidase (*Gpx*) types 1, 3, and 4 were found in both wild-type and $PGF_{2\alpha}$ receptor knockout mice (Figure 3B, lower panel) and no differences in mRNA levels of these enzymes between these two groups were found (figure 3B, top panel).

It was also found that luteal cells express the mRNA for the α-tocopherol transfer protein (*Ttpa*). *Ttpa* mRNA levels were ten-fold higher in luteal cells of $PGF_{2\alpha}$ receptor knockout mice than in cells of wild-type animals (Figure 3B). In wild-type mice, a significant decrease in microsomal glutathione S-transferase (*Mgst*) 2 mRNA levels was found when compared to $PGF_{2\alpha}$ receptor knockout mice, but no differences were found in the expression of *Mgst1* and *Mgst3* (Figure 3B, top panel). *Mgst1* mRNA was found to be expressed at very high relative levels, whereas *Mgst2* and *Mgst3* were expressed at moderate and low relative levels, respectively.

Validation of Microarray Results

To validate our microarray data, semi-quantitative RT-PCR was used. To control for mRNA recovery and reverse transcription efficiency, all RT-PCR experiments were normalized to the housekeeping gene β-actin. RT-PCR data were consistent with microarray results; thus, relative mRNA levels of *Ch25h*, *Akr1c18*, and *Cyp17a1* were significantly higher in wild-type animals. Luteal *Cyp19a1* and *Hsd17b7* relative mRNA levels were significantly higher in $PGF_{2\alpha}$ receptor knockout mice (Figure 4A). Relative messenger RNA levels for *Sod2*, *Sod3*, *Ccs*, *Pdrx6*, *Mgst2*, and *Ttpa* were significantly up-regulated in $PGF_{2\alpha}$ receptor knockout mice (Figure 4B). Fold changes observed by RT-PCR were also consistent with those observed in the microarray experiment. No significant changes in β-actin (*Actb*) mRNA levels were found between samples.

Changes in Luteal Gene Expression between Days 15 and 19 of Pregnancy in Wild-type Animals

Using microarray analysis, we identified several genes that are differentially expressed in the CL of $PGF_{2\alpha}$ receptor knockout and wild-type animals. To further extend these studies and to confirm the participation of these genes in the luteolytic process, we examined their relative expression in the CL of wild-type animals on day 15, which is a fully functional CL, and CL that had already initiated the luteolytic process obtained from mice on day 19 of gestation.

Relative mRNA levels for *Ch25h* were significantly higher on day 19 of pregnancy than on day 15. The opposite scenario was found when relative mRNA levels for *Cyp19a1* and *Hsd17b7* were studied. *Cyp17a1* mRNA was not detectable on day 15 of pregnancy but high levels were found on day 19 (Figure 5).

Higher and statistically significant relative mRNA levels for *Sod2*, *Ccs*, *Prdx6*, *Mgst2*, and *Ttpa* were observed on day 15 of gestation as compared to day 19 of gestation (Figure 5). Lower, but statistically non-significant, *Sod3* mRNA levels were observed on day 19 of pregnancy with respect to day 15. No changes in *Actb* mRNA levels were found between samples.

Western Blot Analysis for cytochrome P450 aromatase (CYP19A1) and superoxide dismutase 2 (SOD2) protein levels in Wild-Type Animals on Days 15 and 19; and in $PGF_{2\alpha}$ Receptor Knockout Mice on Day 19 of Gestation

Next, we used Western Blot to investigate whether changes in mRNA levels of *Cyp19a1* and *Sod2* are reflected in variations in their protein levels. As shown in figure 6, luteal levels of CYP19A1 and SOD2 were low in wild-type mice on day 19 of pregnancy, whereas high levels were found in wild-type animals on day 15 and in $PGF_{2\alpha}$ receptor knockout mice on day 19 of gestation. No changes in β-actin protein (ACTB) levels were found between samples.

Discussion

Our results indicate that during the normal luteolytic process that takes place at the end of pregnancy in rodents there are profound changes in luteal expression of androgen and estrogen synthetic enzymes in addition to the well-known increase in progesterone metabolism. It is likewise interesting that several genes involved in the elimination of free radical species are dramatically down-regulated in luteal cells undergoing luteolysis.

Steroidogenic Genes

The results presented clearly indicate that no changes in luteal mRNA levels of enzymes involved in the uptake of cholesterol or in the synthesis of progesterone take place during luteolysis in mice (Figure 2A). Accordingly, we have found high relative mRNA levels of the scavenger receptor, class B, type 1 (*Scarb1*) both in wild-type and $PGF_{2\alpha}$ receptor knockout mice. SCARB1 binds high-density lipoproteins [14], which are the preferential source of cholesterol in rodents' luteal cells. [15-17], whereas steroidogenic acute regulatory (STAR) [18] and benzodiazepine receptor peripheral-type (BZRP) [19] proteins mediate the transport of cholesterol into the mitochondrion, which is a limiting step in steroid synthesis. We confirmed previous reports showing *Star* and *Bzrp* mRNA expression in the CL [20, 21]. In addition, we demonstrated that in the mouse CL *Star* mRNA expression levels are at least ten times higher than those of

Bzrp; and that the expression of these genes does not differ between wild-type and $PGF_{2\alpha}$ receptor knockout mice. There is evidence indicating that treatment of ewes or cows with $PGF_{2\alpha}$ dramatically reduces concentrations of mRNA encoding *Star* [22, 23]. In pseudopregnant rats, $PGF_{2\alpha}$ has also been shown to reduce *Star* luteal mRNA levels [21]. In contrast, our present results indicate that, in mice, $PGF_{2\alpha}$ does not affect *Star* expression at the end of pregnancy. Direct and indirect evidence support our present finding. $PGF_{2\alpha}$ administration to pregnant rats on day 19 does not affect *Star* mRNA levels (Stocco, unpublished results). Moreover, in mice and rats, total progestin production (progesterone plus 20α-DH-progesterone) remains constant after $PGF_{2\alpha}$ treatment [3], indicating that cholesterol is still supplied without restriction to the cytochrome P450scc further suggesting that luteal expression of *Star* was not affected by $PGF_{2\alpha}$ treatment. This differential effect of $PGF_{2\alpha}$ on *Star* expression is probably due to fundamental differences in the regulation of the CL between species and during pregnancy in rats. In ovine and bovine, LH is the main luteotrophic hormone [24]. In rats, however LH is necessary to sustain luteal function only between days 7 and 10 of pregnancy [25] while placental lactogens are the main luteotrophic hormones during the second part of pregnancy [26].

Interestingly, we found an increase in the mRNA levels of the enzyme cholesterol 25-hydroxylase (*Ch25h*) in wild-type animals. The gene for this enzyme was first cloned in 1998 by Lund *et al.* [27] and the enzyme was shown to cause 25-hydroxylation of cholesterol. Although *Ch25h* is not highly expressed, 25-hydroxycholesterol is a potent regulatory oxysterol and suppresses the activation of transcription factors such as sterol regulatory element binding proteins leading to inhibition of gene transcription [27]. Whether 25-hydroxycholesterol plays any role in the regulation of luteal gene expression is unknown.

In the rat and mouse, a key role controlling progesterone secretion by the CL is played by 20α-hydroxysteroid dehydrogenase (AKR1C18), which converts progesterone into an inactive steroid, 20α-dihydroprogesterone [28, 29]. We confirmed the stimulatory effect of $PGF_{2\alpha}$ on *Akr1c18* expression [3] at the end of pregnancy. A novel finding of this report is that the effect of $PGF_{2\alpha}$ is not limited to an increase in progesterone catabolism, but it also profoundly affects androgen and estrogen synthesis. $P450_{17\alpha}$-

hydroxylase/C17-20 lyase (*Cyp17a1*) is highly expressed in the CL of pregnancy rats until day 12, when it drops to undetectable levels [30]. In agreement with these results, *Cyp17a1* mRNA was not detected on day 15 of gestation. Interestingly, we found high *Cyp17a1* mRNA levels on day 19 of pregnancy, suggesting that luteal expression of this enzyme increases again towards the end of pregnancy. In support of this finding, an increase in ovarian androgen production has been shown at this time [31]. We observed also that *Cyp17a1* mRNA levels are significantly low in $PGF_{2\alpha}$ receptor knockout mice, as compared to wild-type animals. Taken together, these findings suggest that luteal *Cyp17a1* expression increases at the end of pregnancy and that $PGF_{2\alpha}$ action in the CL is required for this to occur. Because it has been demonstrated that administration of dihydrotestosterone, a non-aromatizable androgen, induces abortion [32] and that androstenedione reduces progesterone production in luteal cells [33, 34], we postulate that CYP17A1 may play an important role in the normal progress of luteolysis by increasing intraluteal levels of androstenedione.

Biosynthesis of estrone from androstenedione is catalyzed by cytochrome P450aromatase (CYP19A1). In the rat, the luteal contents of *Cyp19a1* mRNA is low on day 4 of pregnancy, progressively increase reaching maximal levels between days 15 and 19 of gestation, and rapidly decreases to almost undetectable levels on day 23 [35, 36]. The mechanisms that control *Cyp19a1* gene expression in luteal cells, particularly the one or more factors that cause its rapid fall before parturition, are not known. Since we have previously shown that $PGF_{2\alpha}$ administration to pregnant rats on day 19 decreases *Cyp19a1* gene expression [37], and because our present results shows high *Cyp19a1* (mRNA) and CYP19A1 (protein) levels in $PGF_{2\alpha}$ receptor knockout mice with respect to wild-type animals, we postulate that $PGF_{2\alpha}$ is accountable for the dramatic decrease in *Cyp19a1* expression that takes place in the CL of rodents at the end of pregnancy. Finally, not only synthesis of estrone, but also its conversion to the more active estrogen compound, 17β-estradiol, seems to be regulated by $PGF_{2\alpha}$ in luteal cells on day 19 of pregnancy. In agreement with previous studies [38-41], we found that 17β-hydroxysteroid dehydrogenase (*Hsd17b*) type 1 and 7 mRNA levels are high in mouse CL. HSD17B1 and HSD17B7 transform estrone into 17β-estradiol

[40, 42]. It has been shown that luteal *Hsd17b7* mRNA expression rapidly decreases at the end of pregnancy in the rat [43, 44] and mouse [40]. We observed that this decrease does not take place in $PGF_{2\alpha}$ receptor knockout mice.

Antioxidant Genes

As a consequence of normal metabolism, cells generate oxygen radical species. In the particular case of luteal cells, an increase in the generation of ROS has been correlated with decreased progesterone production and cell death [5, 8, 10]. The first reaction of O_2 is a one-electron reduction to superoxide ($O_2^{\cdot}$, Figure 3A). The major defense mechanism against this radical is a family of enzymes named superoxide dismutase (SOD), which catalyze the dismutation of superoxide to H_2O_2. We have shown here that the mouse's CL contains messenger RNA for SOD1, SOD2, and SOD3. Our results show that *Sod2* mRNA is not only highly expressed, but also that its expression is dramatically regulated during pregnancy. We found that in wild-type animals, *Sod2* mRNA and SOD2 protein levels are high on day 15 of pregnancy, but their levels of expression significantly decrease on day 19. Interestingly this decrease in *Sod2* mRNA and SOD2 protein levels does not take place in $PGF_{2\alpha}$ receptor knockout animals. Messenger RNA levels for SOD3 and a protein called copper chaperone for SOD1 (CCS) were also found to be affected by the lack of $PGF_{2\alpha}$ receptor. CCS, by providing Cu(I) to SOD1, is essential for SOD1 activity [45-47]. Evidences suggest that SOD1 plays an important role in ovarian physiology. Thus, it has been shown that Sod1 mRNA antisense decreases luteal progesterone production [48] and that fertility is reduced in *Sod1*$^{-/-}$ mice [49]. However, the mechanisms that control *Sod1* gene expression and/or SOD1 activity in the CL remain to be determined. Our results suggest that luteal SOD1 activity may be regulated indirectly through a decrease in CCS protein expression. These results suggest that at the end of pregnancy, a decrease in SOD2, SOD3 and CCS expression significantly reduces the capacity of luteal cells to cope with superoxide accumulation. Furthermore, $PGF_{2\alpha}$ action in the CL is necessary for the decrease on the expression of these enzymes to occur.

After conversion of $O_2^{\cdot}$ to H_2O_2, the next step is the reduction of H_2O_2 to water. Several enzymes catalyze this reaction, including catalase, glutathione peroxidase (GPX), and a relative newly-discovered family of antioxidant proteins called peroxiredoxins (PRDX) [50]. We have found that the mouse CL contains mRNA for each one of these enzymes. Noteworthy is that mRNA levels for catalase found in the CL at the end of pregnancy are low when compared to those of the Prdx family, suggesting that PRDX, but not catalase, may play a more important role in the elimination of hydrogen peroxide in luteal cells. It has been recently postulated that PRDX6 eliminates H_2O_2 and reduces the number of hydroxyl radicals produced by the Fenton reaction and the subsequent tissue injury [51]. Consistent with this finding, *Prdx*$^{-/-}$ mice were shown to have more severe tissue damage and higher mortality rates than controls, even when catalase (CAT) or GPX proteins were normally expressed [51]. In addition, macrophages from the *Prdx*$^{-/-}$ mutants contain more H_2O_2 and are more susceptible to oxidant-induced cell death than macrophages of wild-type animals, indicating that PRDX6 functions *in vivo* as an antioxidant and non-redundantly with respect to other PRDX and antioxidant enzymes. We have found that *Prdx6* mRNA levels are highly in non-luteolytic CL (day 15 wild-type and day 19 $PGF_{2\alpha}$ receptor knockout animals), suggesting that a drop in *Prdx6* expression may be important for the normal evolution of the luteolytic process. To our knowledge this is the first report on the expression and regulation of *Prdx6* mRNA levels in luteal cells.

The hydroxyl radical is the most reactive free radical [52]. It could be formed *in vivo* from superoxide and/or from hydrogen peroxide via the Fenton reaction (Figure 3A). Hydroxyl radicals immediately react with surrounding target molecules at the site where they are generated, especially with polyunsaturated fatty-acid-producing lipid hydroperoxides (L-OO$^{\cdot}$). Lipid hydroperoxides can then react with other lipids, propagating their own formation. This chain reaction leads to membrane damage. Protection against lipid peroxidation is given in part by α-tocopherol, which is highly concentrated in luteal cells [8, 53]. Reduced α-tocopherol is then regenerated by ascorbic acid. We have found that luteal cells express the mRNA for α-tocopherol transfer protein (TTPA), which is a cytosolic protein that in the liver selectively transfers α-

tocopherol from lipoproteins taken up by hepatocytes via the endocytic pathway to newly-secreted lipoproteins [54]. Interestingly, luteal cells undergoing luteolysis contain significantly lower levels of *Ttpa* mRNA levels than functional luteal cells, suggesting that TTPA may play an important role in the maintenance of normal luteal function; probably TTPA facilitates the accumulation of α-tocopherol in luteal membranes. Microsomal glutathione S-transferase (MGST2) 2 is also involved in scavenging lipid soluble radicals [55, 56]. MGST2 location, bound to the membrane of the endoplasmic reticulum where several steroidogenic enzymes are localized, suggests that lipid peroxides formed because the high rate of steroid synthesis in luteal cells may be efficiently eliminated by MGST2. Our results indicate that a decrease in the expression of this enzyme may be important during the luteolytic process in rodents.

As mentioned earlier, it is well known that hydrogen peroxide, superoxide anion, and lipid peroxides are generated during the luteolytic process [4-10], however, the events that lead to this accumulation of ROS are largely unknown. Our results have shed light upon a possible mechanism by which this increase in ROS production takes place during luteolysis. It is proposed that $PGF_{2\alpha}$, by decreasing the expression of free radical scavenger proteins, facilitates the accumulation in ROS, which in turn initiates a cascade of events that will finally cause luteal cell death.

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Figure Legends

Figure 1. Expression profiling of 45k genes between the CL of wild-type and $PGF_{2\alpha}$ receptor knockout mice. For each gene, average expression levels (in arbitrary units) were calculated from four independent experiments and displayed on a scatter plot. * Pearson correlation coefficient + SEM, n=4.

Figure 2. (A) A simplified scheme of the cholesterol and steroid metabolism in mouse luteal cells. (B) The top graph represents the ratio of the luteal expression of steroidogenic genes in wild-type and $PGF_{2\alpha}$ receptor knockout mice. The scale indicates the values of expression as a ratio between wild-type/$PGF_{2\alpha}$ receptor knockout animals (higher expression in wild type) or $-1 \times PGF_{2\alpha}$ receptor knockout/wild type for negative values (higher expression in knockout). The bottom graph reports the relative expression of these genes against the housekeeping gene β-actin. Bars represent the average of four different experiments + standard error of the mean. *Denote genes whose expression is significantly different between wild-type and $PGF_{2\alpha}$ receptor knockout mice ($p < 0.05$; ANOVA I).

Figure 3. (A) A simplified scheme of ROS metabolism. ($^{\bullet}$) indicate free radical. (B) The top graph represents the negative ratio of the luteal expression of genes involved in ROS metabolism in $PGF_{2\alpha}$ receptor knockout and wild-type animals. The bottom graph reports the relative expression of these genes against the housekeeping gene β-actin. Bars represent the average of four different experiments + standard error of the mean. *Denote genes whose expression is significantly different between wild-type and $PGF_{2\alpha}$ receptor knockout mice ($p < 0.05$; ANOVA I).

Figure 4. Reverse transcription analysis of steroidogenic genes (A) and of genes involved in ROS metabolism (B) that were found to be significantly different between wild-type (+/+) and $PGF_{2\alpha}$ receptor knockout (–/–) mice. The relative expression columns on the left represent the densitometric analysis of each band, expressed as a ratio between the intensity of each gene and β-actin (average and SEM, n=4). $*p < 0.05$ and $**p < 0.01$ (Student *t* test). The ratio column represents the ratio between the relative intensity of each gene on –/– and +/+ animals.

Figure 5. Expression of steroidogenic and antioxidant genes in wild-type mouse CL on day 15 and day 19 of pregnancy. The relative expression columns on the left represent the densitometric analysis of each band, expressed as a ratio between the intensity of each gene and β-actin (average and SEM, n=3). $*p < 0.05$ and $** p < 0.01$ (Student *t* test). The ratio column represents the ratio between the relative intensity of each gene on days 15 and 19 of pregnancy.

Figure 6. Cytochrome P450 Aromatase (CYP19A1), Superoxide Dismutase 2 (SOD2), and β-actin (ACTB) protein expression levels of wild-type (+/+) animals on day 15 and 19 of pregnancy and of $PGF_{2\alpha}$ receptor knockout (–/–) mice on day 19 of gestation. Whole corpora lutea proteins were separated by SDS-PAGE transferred to nitrocellulose, and immunoblotted with specific CYP19A1, SOD2 or ACTB antiserum. Blots are representative of two different sets.

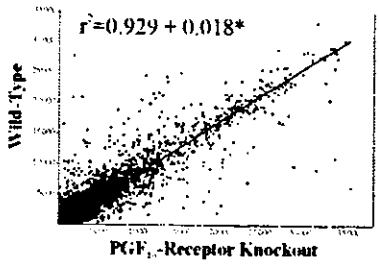

Figure 1

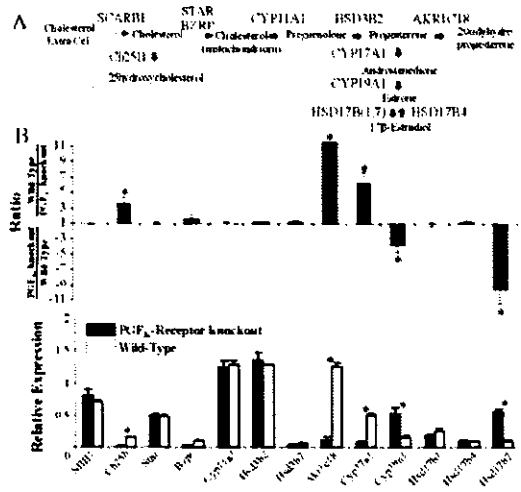
A
B
Ratio
Relative Expression
PGF2α-Receptor knockout
Wild-Type

Figure 2

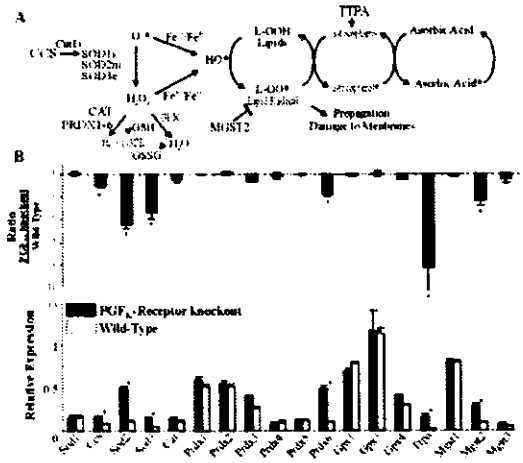
A
TTPA
Ascorbic Acid
Propagation
Damage to Membranes
B
Ratio
PGF2α-Receptor knockout
Wild-Type
Relative Expression

Figure 3