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$G_{q/11}$-Dependent Changes in the Murine Ovarian Transcriptome at the End of Gestation[1]

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ABSTRACT

Parturition in rodents is highly dependent on the engagement of the luteal prostaglandin F2 alpha receptor, which, through activation of the $G_{q/11}$ family of G proteins, increases the expression of *Akr1c18*, leading to an increase in progesterone catabolism. To further understand the involvement of $G_{q/11}$ on luteolysis and parturition, we used microarray analysis to compare the ovarian transcriptome of mice with a granulosa/luteal cell-specific deletion of $Galpha_{q/11}$ with their control littermates on Day 18 of pregnancy, when mice from both genotypes are pregnant, and on Day 22, when mice with a granulosa/luteal cell-specific deletion of $Galpha_{q/11}$ are still pregnant but their control littermates are 1–2 days postpartum. Ovarian genes up-regulated at the end of gestation in a $Galpha_{q/11}$-dependent fashion include genes involved in focal adhesion and extracellular matrix interactions. Genes down-regulated at the end of gestation in a $Galpha_{q/11}$-dependent manner include *Serpina6* (which encodes corticosteroid-binding globulin); *Enpp2* (which encodes autotaxin, the enzyme responsible for the synthesis of lysophosphatidic acid); genes involved in protein processing and export; reproductive genes, such as *Lhcgr*; the three genes needed to convert progesterone to estradiol (*Cyp17a1*, *Hsd17b7*, and *Cyp19a1*); and *Inha*. Activation of ovarian $G_{q/11}$ by engagement of the prostaglandin F2 alpha receptor on Day 18 of pregnancy recapitulated the regulation of many but not all of these genes. Thus, although the ovarian transcriptome at the end of gestation is highly dependent on the activation of $G_{q/11}$, not all of these changes are dependent on the actions of prostaglandin F2 alpha.

$G_{q/11}$, *parturition*, *transcriptome*

INTRODUCTION

It is now clear that at least two families of G proteins, G_s and $G_{q/11}$, are essential to maintain the functions of granulosa and luteal cells. G_s activation by the luteinizing hormone (LH) receptor in granulosa cells is required for initiating the events leading to the resumption of meiosis and the expansion of cumulus cells, but it is also required for the induction of the progesterone receptor (*Pgr*) and subsequent rupture of the ovarian follicles [1, 2]. The activation of $G_{q/11}$ by the LH receptor in granulosa cells is not required for the resumption of meiosis and the expansion of cumulus cells [3]. Together with the LH receptor-activated G_s, however, the LH receptor-activated $G_{q/11}$ is required for optimal induction of the granulosa cell *Pgr*, follicular rupture, and an optimal ovulatory response [1, 3]. Thus, genetic deletion of $G\alpha_{q/11}$ from granulosa cells compromises the LH receptor-induced expression of the granulosa cell *Pgr* and ovulation [3].

Studies with mice harboring a granulosa/luteal cell-specific deletion of $G\alpha_{q/11}$ have demonstrated that the differentiation of granulosa cells into luteal cells, as well as the establishment and maintenance of pregnancy, does not require $G_{q/11}$ [3, 4]. Parturition, on the other hand, is dependent on the prostaglandin F2α (PGF2α)-provoked activation of luteal $G_{q/11}$ [4]. A conditional deletion of $G\alpha_{q/11}$ from granulosa/luteal cells disrupts the actions of PGF2α on luteal cells that normally occur toward the end of gestation [4]. The ovaries of pregnant mice with a conditional deletion of $G\alpha_{q/11}$ from granulosa/luteal cells display a defect in the PGF2α-provoked induction of *Ark1c18*, the gene encoding for 20α-hydroxysteroid dehydrogenase, the enzyme that catabolizes progesterone to 20α-hydroxyprogesterone [4]. As such, the mice exhibit a delay or failure of parturition because they do not experience the reduction in progesterone levels that precipitate parturition [4].

The crucial role of luteal cell $G\alpha_{q/11}$ in the expression of luteal *Ark1c18* at the end of gestation prompted us to examine the hypothesis that luteal cell $G_{q/11}$ is also involved in the expression of other ovarian genes that may participate in luteolysis and parturition. This is an important question, because in addition to activating $G_{q/11}$, the PGF2α receptor also activates $G_{12/13}$ [5], and because there are other hormones, such as oxytocin [6, 7] or lysophosphatidic acid [8, 9], that can activate luteal $G_{q/11}$. To test this hypothesis we compared the ovarian transcriptome of mice with a granulosa/luteal cell-specific deletion of $G\alpha_{q/11}$ with the transcriptome of their control littermates on Days 18 and 22 of pregnancy. The results presented show that changes in the ovarian transcriptome that occur at the end of gestation (Day 22 of pregnancy, when mice lacking $G\alpha_{q/11}$ still have not given birth but their control littermates have) are highly dependent on $G\alpha_{q/11}$. Not all of the $G\alpha_{q/11}$-dependent genes are dependent on the actions of PGF2α, however.

MATERIALS AND METHODS

Mice

A colony of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice were generated by crossing $G\alpha_q^{f/f};G\alpha_{11}^{-/-}$ mice and *Cyp19Cre* transgenic mice as previously described [3]. The colony of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice was maintained by crossing $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ males with $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ females [4]. The resulting $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ females were used as experi-

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mental and control animals, respectively. Genotyping was performed using tail genomic DNA and PCR amplification as described previously [10–14].

Adult females (ages 6–10 wk) were synchronized on estrus by injecting them with progesterone and cloprostenol [15], housed overnight with males of proven fertility, and checked the following morning for vaginal plugs. Day 1 of pregnancy (1 dpc) was counted as the morning when a vaginal plug was found, and the pregnant animals were killed in the morning on Days 18 or 22 of pregnancy as indicated. In some experiments pregnant mice (17 dpc) were injected subcutaneously with vehicle only or 1 μg of cloprostenol, a PGF2α agonist [16], and killed 24 h later for the preparation of ovarian RNA. All animal procedures were approved by the Institutional Animal Care and Use Committee for the University of Iowa.

cAMP Assays

Luteal cells were prepared from the ovaries of mice on 18 or 22 dpc exactly as described previously for pseudopregnant ovaries [4]. The freshly isolated luteal cells were immediately suspended in 500 μl of Dulbecco modified Eagle medium/F12 supplemented with 10 mM Hepes, bovine serum albumin (1 mg/ml), and 1 mM isobutyl methylxanthine and incubated without or with human chorionic gonadotropin (hCG; 100 ng/ml), for 30 min at 37°C. Total cAMP (cells + medium) was then extracted and quantitated as described earlier [17–19], except that we used a commercially available enzyme immunoassay kit from Cayman Chemical instead of a radioimmunoassay. All samples were assayed in a single assay and the intraassay coefficient of variation was 10%.

Microarray Analysis

Transcription profiling was performed in triplicate (n = 3) for each genotype on 18 dpc and 22 dpc, respectively. The profiling was done using ovarian RNA purified from the ovaries and the mouse WG-6 V2.0 Illumina platform. All steps of the transcription profiling (except for the isolation of the RNA) and analyses were performed by the Iowa Institute of Human Genetics. Gene enrichment analysis was carried out using publicly available software (http://bioinfo.vanderbilt.edu/webgestalt).

RNA Isolation and Real-Time PCR

Ovarian RNA was prepared as described earlier [4]. Target and control gene (*Gapdh*) expressions were measured by quantitative PCR (qPCR) using 5–50 ng of purified RNA, the iTaq Universal SYBR Green One Step Kit (Bio-Rad Laboratories), and a CFX90 real-time PCR detection system (Bio-Rad Laboratories). Each experimental group included a minimum of five replicate animals (see Figure legends), and each sample was run in duplicate on the qPCR reactions. Data were quantitated by the comparative C_q method as described previously [20], using *Gapdh* as control or normalizer gene, and are always expressed relative to a control group as indicated in each of the figures. There was no statistical difference among the average quantification cycles (C_q) for the control gene (*Gapdh*) for any group or condition.

The primers used were as follows: *Lhcgr*: forward, CCTTGTGGGTGTCAGCAGTTA; reverse, TTGTGACAGAGTGGATTCCACAT; *Cyp19a1*: forward, GACGGGCCCTGGTCTTGT; reverse, CCGGTCCAAATGCTGCTT; *Cyp11a1*: forward, CCCGGAGCGGTTCCTT; reverse, CCAATGGGCCTCTGATAATACTG; *Cyp17a1*: forward, TGGAGGCCACTATCCGAGAA; reverse, CACATGTGTGTCCTTCGGGA; *Inha*: forward, TGTGGGTAAAGTGGGGGAGA; reverse, GTCTTTGGTGGTGTCTGCGA; *Hsd17b7*: forward, AGGCGTCGTGATGACCAATA; reverse, AAAAAGCGAAGGAGCCACATT; *Enpp2*: forward, GGGCTGCACCTGTGATGATA; reverse, GATGTCTCTCTTCTGTAGATCCTT; *Serpina6*: forward, TTCCAAGGCAGATGAACCTGTA; reverse, GTCTTTGGTGGTGTCTGCGA; *Wfs1*: forward, TCGTCAGCAGTGAATCCAAGAA; reverse, AATGATGCCCTTGGCGTACT; *Jun*: forward, CTTGTGCCCCAAGAACGTG; reverse, GTGACACTGGGAAGCGTGTT; *Tnc*: forward, CAATAACCACAGTCAGGGCGT; reverse, CCCTGGAATTTATGCCCGCT; *Ssr4*: forward, TGGCTCTTTATGCCGACGTT; reverse, AGGCTCCAGGACACCTGATA; and *Gapdh*: forward, GACGGCCGCATCTTCTTG; reverse, ACCGACCTTCACCATTTTGTCT.

Hormone Assays

Serum estradiol and Inhibin A concentrations were measured using ELISA by the Ligand Assay and Analysis Core of the University of Virginia. The measurable ranges for estradiol and inhibin A are 3–300 pg/ml and 10–950 pg/ml, respectively. The intraassay and interassay coefficients of variation for estradiol are 6.1% and 8.9%, respectively, and the corresponding values for Inhibin A are 1.5% and 4.1%.

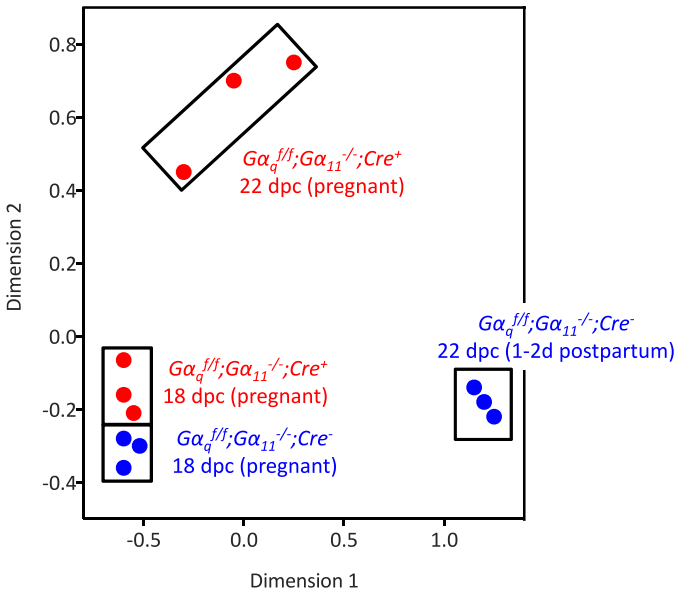

FIG. 1. Multidimensional plot of ovarian gene expression in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice on 18 and 22 dpc. The blue and red circles enclosed in the rectangles highlight the overall similarity of gene expression in the individual ovaries of the four groups of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice analyzed on 18 and 22 dpc as indicated. On 18 dpc $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are pregnant. On 22 dpc the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are still pregnant but the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice are 1–2 days postpartum.

RESULTS

Microarray Analysis

Ovarian RNA was prepared *from* $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ females on 18 dpc (three mice of each genotype) and 22 dpc (three mice of each genotype). On 18 dpc, mice from both genotypes are pregnant. Because the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice give birth on 20–21 dpc, they are 1–2 days postpartum on 22 dpc. The $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ are still pregnant on 22 dpc because of the previously described delay or failure of parturition [4]. The 12 RNA samples were subjected to expression profiling using the mouse WG-6 V2.0 Illumina platform. Array data were imported using the Bead array software package (http://www.bioconductor.org/packages/release/bioc/html/beadarray.html) and were quantile normalized. Differential expression was determined using the Limma software package (http://bioconductor.org/packages/release/bioc/html/limma.html).

A multidimensional scale plot of the microarray data (Fig. 1) clearly shows that based on the similarity of gene expression, the 12 mice used for the microarray analysis can be segregated into 4 groups according to genotype and length of gestation. Ovarian gene expression was rather similar between the two genotypes on 18 dpc (when mice from both genotypes are pregnant) but was very different on 22 dpc, when the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice have given birth but the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice have not. Also, the three mice of each length of gestation and genotype clustered very close to each other, but there was more variability in the group of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice on 22 dpc than in any of the other three groups.

Because we used 12 mice and there are a large number of probes represented in the mouse WG-6 V2.0 Illumina chips (~45 000), the results of the differential expression can be analyzed using rather stringent criteria for statistical significance ($P < 0.001$). A comparison of gene expression

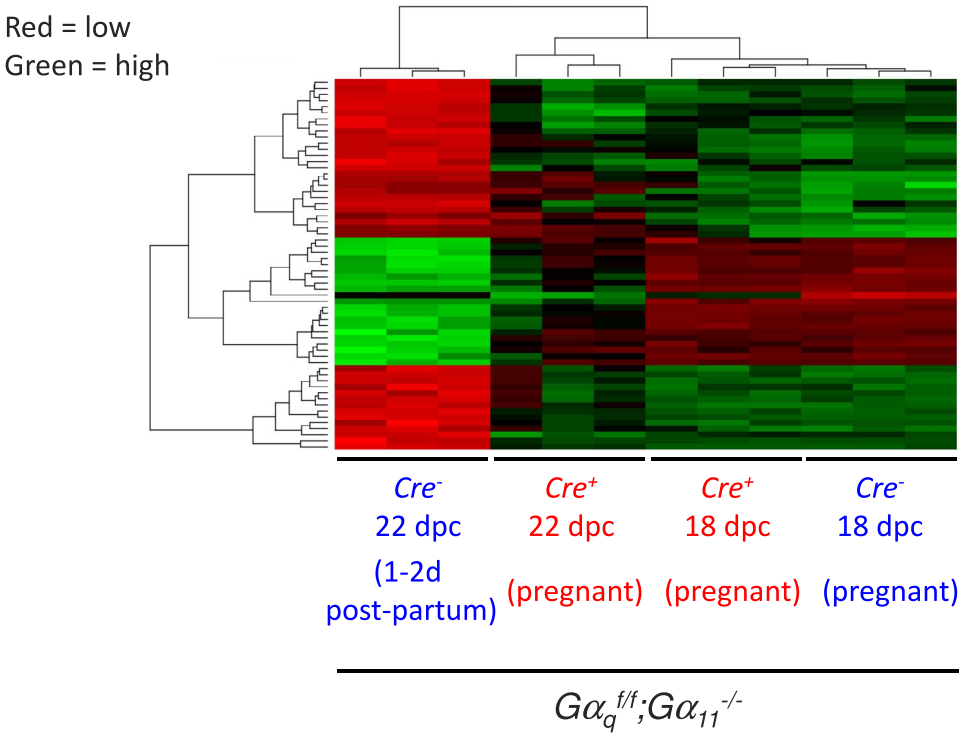

FIG. 2. Heat map of ovarian gene expression in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice on 18 and 22 dpc. The probes included in this heat map were first selected by comparing gene expression in the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice on 22 and 18 dpc. On 18 dpc $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are pregnant. On 22 dpc the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are still pregnant but the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice are 1–2 days postpartum. Only those probes that were differentially expressed with a $P < 0.001$ were chosen. This list was sorted again based on the extent of the differential expression, and only those probes that were differentially expressed by at least two orders of magnitude (100-fold) are shown. An increase in expression is shown by the different shades of green, and a decrease in expression is shown by the different shades of red. The clusters shown contain duplicate probes and probes for unknown genes. The identity of the up- and down-regulated genes (after removal of duplicate probes and probes for unknown genes) is shown in Tables 1 and 2.

between the two genotypes on 18 dpc revealed no differentially expressed probes, either when using this criterion or when a more relaxed ($P < 0.005$) level of significance was used. A comparison of gene expression in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice on 22 and 18 dpc using $P < 0.001$ generated a list of 288 differentially expressed probes. This group of probes was then ranked to include only probes that were differentially expressed by at least 100-fold (2 log-fold), to generate the heat map presented in Figure 2. As expected from the above discussion, gene expression was generally similar in the ovaries of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice on 18 dpc. This map also clearly shows two clusters of probes (shown in red, about 40 probes) that were down-regulated in the ovaries of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ on 22 dpc, and one cluster of probes (shown in green, about 20 probes) that was up-regulated in the ovaries of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice on 22 dpc. The down- or up-regulation of these clusters of probes was not obvious in the ovaries of the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice on 22 dpc, however.

Manually removing duplicate probes and probes for unknown genes further reduced the up-regulated cluster to the 11 genes shown in Table 1. This table shows that the magnitude of the increased expression of the 11 genes in the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice was much lower or absent compared with the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice (compare the log of fold increase in the Cre^-/Cre^- and Cre^+/Cre^+ columns) and, with the exception of *Akr1c18* and *Chrna1*, the up-regulation of the other genes in the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice was no longer statistically significant (adjusted P value <0.001 in the Cre^-/Cre^- column and >0.001 in the Cre^+/Cre^+ column). The same type of analysis reduced the down-regulated clusters to the 28 genes shown in Table 2. Again, the decreased expression of these genes was much lower or absent (compare the log of fold decrease in the Cre^-/Cre^- and Cre^+/Cre^+ columns) and no longer statistically significant in the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice (adjusted P value <0.001 in the Cre^-/Cre^- column and >0.001 in the Cre^+/Cre^+ column).

TABLE 1. Ovarian genes up-regulated at the end of gestation in a $G\alpha_{q/11}$-dependent fashion by at least two orders of magnitude and $P < 0.001$.

	22 dpc/18 dpc[a]			
	Log of fold increase		Adjusted P value	
Symbol	Cre^-/Cre^-	Cre^+/Cre^+	Cre^-/Cre^-	Cre^+/Cre^+
Akr1c18[b]	7.08	5.54	1.3E-05	8.0E-04
Tnc[b]	3.73	0.01	4.1E-05	1.0E+00
Cdkn2b	3.70	2.23	3.4E-05	8.7E-03
Onecut2	3.63	0.66	8.9E-05	6.8E-01
Rgl1	3.20	1.10	6.3E-06	3.9E-02
Bhlhb2	2.86	0.89	9.7E-07	2.1E-02
Sbsn	2.75	1.24	5.9E-05	5.2E-02
Slc46a3	2.72	0.33	1.2E-06	5.1E-01
Gsg1l	2.45	−0.02	2.7E-05	9.8E-01
Chrna1	2.42	1.18	3.2E-07	8.0E-04
Cyp4f14	2.28	0.10	1.1E-07	9.1E-01

[a] On 18 dpc $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are pregnant. On 22 dpc the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are still pregnant but the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice are 1–2 days postpartum.
[b] These genes were chosen for further analysis as discussed in the text.

TABLE 2. Ovarian genes down-regulated at the end of gestation in a $G\alpha_{q/11}$-dependent fashion by at least two orders of magnitude and $P < 0.001$.

	22 dpc/18 dpc[a]			
	Log of fold decrease		Adjusted *P* value	
Symbol	Cre^-/Cre^-	Cre^+/Cre^+	Cre^-/Cre^-	Cre^+/Cre^+
Serpina6[b]	−6.87	−1.04	9.2E-09	4.8E-02
Enpp2[b]	−5.52	−0.64	4.1E-08	2.3E-01
Cyp19a1[b]	−5.26	−0.73	6.2E-09	4.8E-02
Abp1	−4.12	0.36	5.9E-05	9.3E-01
Cyp17a[b]	−3.39	0.13	3.4E-04	9.9E-01
Grp	−3.21	0.03	6.0E-04	9.8E-01
Bace2	−3.06	−0.25	1.4E-04	7.0E-01
Hsd17b[b]	−2.95	−0.39	3.7E-05	8.0E-01
Apof	−2.94	−0.39	5.3E-04	9.1E-01
Itih4	−2.93	−0.37	2.2E-05	7.6E-01
Kng1	−2.91	0.00	2.7E-05	1.0E+00
Slc7a4	−2.88	−0.09	3.4E-05	8.1E-01
Sdk1	−2.81	−0.70	2.7E-05	2.7E-01
Cryba4	−2.76	0.46	5.9E-06	3.9E-01
Lrp11	−2.74	−0.67	1.4E-04	5.1E-01
Acot3	−2.66	−0.86	3.7E-04	3.7E-01
Fkbp11	−2.59	−0.67	2.2E-05	2.2E-01
Rasgrp1	−2.49	0.04	6.9E-04	9.7E-01
Elovl6	−2.44	−0.15	2.2E-04	9.0E-01
Mt3	−2.38	−0.05	5.7E-04	9.9E-01
Slc4a4	−2.37	0.02	4.0E-04	9.9E-01
Inha[b]	−2.23	0.59	6.7E-06	1.1E-01
Gldn	−2.20	0.00	9.9E-05	1.0E+00
Lhcgr[b]	−2.20	−0.22	3.4E-05	9.3E-01
Lrrn3	−2.10	−1.41	1.6E-05	2.7E-03
Gng12	−2.10	−0.23	3.8E-05	1.5E-01
Slc41a3	−2.04	0.75	9.7E-05	1.5E-01
Prr15	−2.04	−0.18	5.7E-04	9.6E-01

[a] On 18 dpc $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are pregnant. On 22 dpc the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are still pregnant but the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice are 1–2 days postpartum.
[b] These genes were chosen for further analysis as discussed in the text.

We next used pathway enrichment software to categorize the 138 probes that were individually up-regulated as well as the 150 probes that were individually down-regulated ($P < 0.001$) into Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Using the default criteria for this analysis (at least two genes per group and $P < 0.01$ for the group) we identified 34 up-regulated genes that can be grouped into 8 KEGG pathways (Table 3) and 49 down-regulated genes that can be grouped in 7 KEGG pathways (Table 4).

The entire microarray data set is included herein (MS Excel file) as Supplemental Table S1 (available online at www.biolreprod.org).

Validation of the Microarray Analysis by qPCR

Akr1c18 was the gene with the highest magnitude of $G\alpha_{q/11}$-dependent up-regulation (Table 1), and the validation of this result by qPCR has already been accomplished elsewhere [4]. We chose to further analyze two up-regulated genes, *Tnc* and *Jun*, based on a combination of the magnitude of up-regulation (Table 1) and their grouping into the ''focal adhesion'' KEGG pathway (Table 3). We also chose to further analyze nine down-regulated genes based on the magnitude of down-regulation (*Serpina6* and *Enpp2*; Table 2), their known involvement in reproductive functions (*Cyp19a1*, *Cyp17a1*, *Hsd17b7*, *Lhcgr*, and *Inha*; Table 2), their grouping into a KEGG pathway (*Wfs1* and *Ssr4* in the ''protein processing in endoplasmic reticulum'' KEGG pathway in Table 4), or a combination of all three criteria—magnitude of down-regulation, their known involvement in reproductive functions, and their grouping into the ''steroid hormone biosynthesis'' KEGG pathway (*Cyp19a1*, *Cyp17a1*, and *Hsd17b7*; Tables 2 and 4).

TABLE 3. Pathway enrichment analysis of ovarian genes that are up-regulated at the end of gestation in a $G\alpha_{q/11}$-dependent fashion.

KEGG pathways[a]	Enrichment ratio	Adjusted *P* value
Metabolic pathways	4.23	0.0012
Cyp4f18		
Sgms2		
Grhpr		
Cmbl		
Pank1		
Nags		
Pmm1		
Cyp4f14		
Acss2		
Acsl4		
Fdps		
Focal adhesion	11.39	0.0012
Tnc[b]		
Vegfb		
Ppp1cb		
Jun[b]		
Cav2		
Peroxisome proliferator receptor signaling	17.08	0.0033
Scd1		
Sorbs1		
Acsl4		
Complement and coagulation cascades	17.98	0.0033
C1qc		
C1qb		
F13a1		
Bacterial invasion of epithelial cells	19.25	0.0033
Elmo1		
Cav2		
Gab1		
Extracellular matrix receptor interaction	15.89	0.0036
Sdc1		
Tnc[b]		
Sdc3		
Endocytosis	8.28	0.0047
Ccr5		
Rab11fip5		
Cav2		
Cytokine-cytokine receptor interaction	7.44	0.0062
Ccr5		
Vegfb		
Cx3cr1		
Il10rb		

[a] Gene symbols identified as being enriched for a given pathway are shown underneath the KEGG pathway name.
[b] These genes were chosen for further analysis as discussed in the text.

Quantitative PCR validation of the microarray data for the up-regulated (Fig. 3) and down-regulated (Figs. 4 and 5) genes on 18 and 22 dpc clearly recapitulated the microarray data. Their expression increased (Fig. 3) or decreased (Figs. 4 and 5) at the end of gestation in the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$, mice but the magnitude of this change was blunted or absent in the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice.

Effect of a PGF2α Receptor Agonist on the Expression of Luteal $G\alpha_{q/11}$-Dependent Genes

The engagement of the PGF2α receptor by uterine-derived PGF2α is recognized as the main determinant of luteolysis and

TABLE 4. Pathway enrichment analysis of ovarian genes that are down-regulated at the end of gestation in a $G\alpha_{q/11}$-dependent fashion.

KEGG pathway[a]	Enrichment ratio	Adjusted *P* value
Protein processing in endoplasmic reticulum	36.42	9.99E-18
Rpn2		
Ssr3		
P4hb		
Ssr2		
H47		
Wfs1[b]		
Ssr4[b]		
Sec61a1		
Ddost		
Hyou1		
Dad1		
Sec13		
Lman2		
Yod1		
Sec61g		
Metabolic pathways	6.93	2.90E-10
Cyp19a1[b]		
Cyp17a1[b]		
Ddost		
Hsd17b7[b]		
Akr1b7		
Dad1		
Hsd11b1		
Cds1		
Pgs1		
Ndufa1		
Pycr2		
Rpn2		
Cmas		
Alg2		
Pla2g12a		
Adpgk		
Alg3		
Mgll		
Aldh3a1		
Bcat2		
N-glycan biosynthesis	41.04	2.27E-06
Alg3		
Rpn2		
Dad1		
Alg2		
Ddost		
Steroid hormone biosynthesis	29.84	0.0001
Cyp19a1[b]		
Hsd17b7[b]		
Cyp17a1[b]		
Hsd11b1		
Protein export	38.47	0.0006
Spcs3		
Sec61g		
Sec61a1		
Metabolism of xenobiotics by cytochrome P450	15.99	0.0044
Aldh3a1		
Gstm5		
Gstk1		
Glycerophospholipid metabolism	15.93	0.0044
Pgs1		
Cds1		
Pla2g12a		

[a] Gene symbols identified as being enriched for a given pathway are shown underneath the KEGG pathway name.
[b] These genes were chosen for further analysis as discussed in the text.

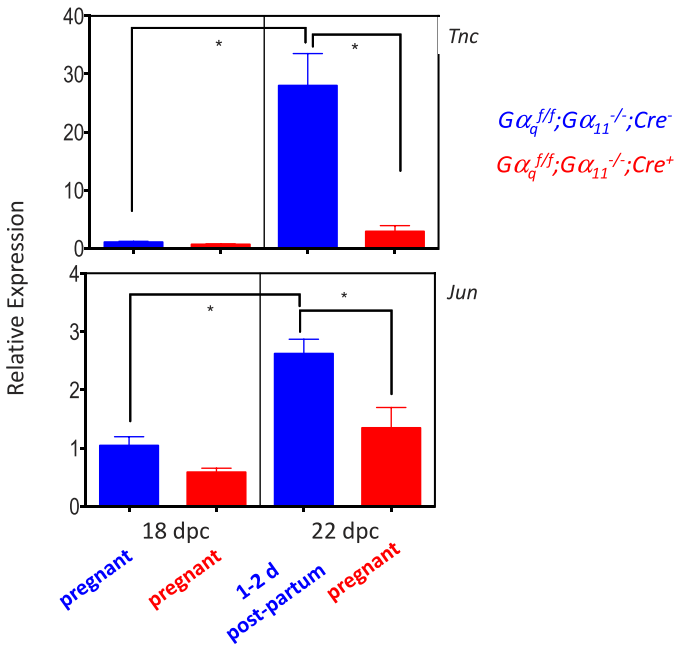

FIG. 3. Quantitative PCR validation of the changes in expression of ovarian *Tnc* and *Jun* at the end of gestation in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice. For each gene, data are expressed relative to the gene's expression in the ovaries of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice on 18 dpc (blue bar on the left panel of each graph). Each bar is the mean ± SEM of five different animals analyzed on the indicated days. Statistically significant differences ($P < 0.05$) were calculated using ANOVA and are shown by the brackets and asterisks. On 18 dpc $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are pregnant. On 22 dpc the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are still pregnant but the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice are 1–2 days postpartum.

parturition in rodents [4, 21–29], and the increased expression of one of the genes found in the microarray (*Akr1c18*; Table 1) has already been shown to be dependent on the activation of $G_{q/11}$ by the PGF2α receptor [4, 26, 27]. To determine whether PGF2α also influenced the expression of the 11 $G\alpha_{q/11}$-dependent genes shown in Figures 3–5, we injected mice of both genotypes with cloprostenol (a PGF2α agonist) on 17 dpc and analyzed ovarian gene expression 24 h later. Figure 6 shows that the expression of only one (*Jun*) of the two chosen up-regulated genes was increased when pregnant $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice were injected with cloprostenol, and that this response was absent in the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice. The expression of ovarian *Tnc* was not affected by cloprostenol in either genotype.

The effect of cloprostenol on the down-regulated genes was also mixed (Figs. 7 and 8). This PGF2α agonist decreased the expression of ovarian *Serpina6*, *Ssr4*, *Enpp2*, *Lhcgr*, *Hsd17b7*, and *Cyp19a1* in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice but not in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice. Cloprostenol had no effect on the expression of ovarian *Wfts1*, *Cyp17a1*, or *Inha* in either mouse genotype.

Physiological Correlates of the Gαq/11-Dependent Changes in Reproductive Gene Expression That Occur at the End of Gestation

The decrease in expression of steroidogenic enzyme genes and the *Lhcgr* that occurred at the end of gestation (Table 2 and Fig. 5) implies that the decline in progesterone and estradiol levels that occur during this time period [27, 30] is coordinately regulated in a $G\alpha_{q/11}$-dependent fashion. The dependency of the decline in progesterone on $G\alpha_{q/11}$ has

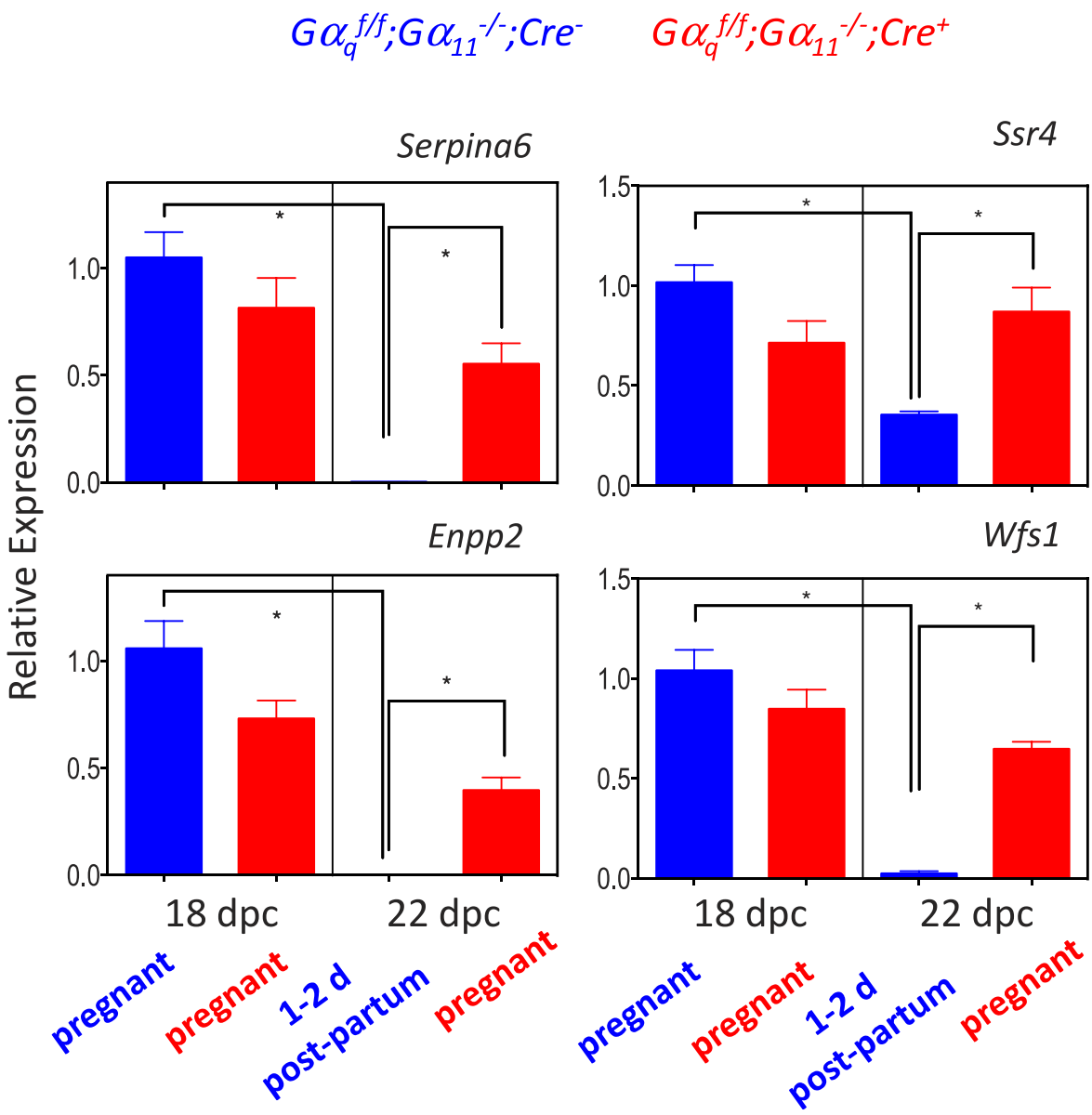

FIG. 4. Quantitative PCR validation of the changes in expression of ovarian *Serpina6*, *Enpp2*, *Wfs1*, and *Ssr4* at the end of gestation in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice. For each gene, data are expressed relative to the gene's expression in the ovaries of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice on 18 dpc (blue bar on the left panel of each graph). Each bar is the mean ± SEM of 5–10 different animals analyzed on the indicated days. Statistically significant differences ($P < 0.05$) were calculated using ANOVA and are shown by the brackets and asterisks. Some bars are not visible because of the scale used. On 18 dpc $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are pregnant. On 22 dpc the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are still pregnant but the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice are 1–2 days postpartum.

already been documented [4]. Figure 9A shows that estradiol levels in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice on 18 dpc were similar and that they declined on 22 dpc in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice but remained elevated in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice. Figure 9B similarly shows that the responsiveness of luteal cells to LH/CG was similar in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice on 18 dpc, and that it declined on 22 dpc in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice but remained elevated in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice. Lastly, the data in Figure 9C show that circulating levels of Inhibin A, another ovarian product, follow the same pattern predicted by the changes in the ovarian expression of *Inha* documented in Table 2 and Figure 5.

DISCUSSION

The murine parturition pathway is initiated by the increased expression of the *Ark1c18* gene in luteal cells. This gene encodes for 20α-hydroxysteroid dehydrogenase an enzyme that inactivates progesterone by converting it to 20α-hydroxyprogesterone. In pregnant rodents the expression of luteal *Akr1c18* is initially silenced by the actions of prolactin and placental lactogens [29, 31–38]. As pregnancy progresses, expression of the luteal PGF2α receptor increases [21–24], and it is engaged by uterine-derived PGF2α [25], which, through the activation of $G_{q/11}$ [4], increases the expression of the *Ark1c18* gene and the conversion of progesterone to 20α-hydroxyprogesterone [26–29]. The resulting drop in progesterone ultimately allows uterine

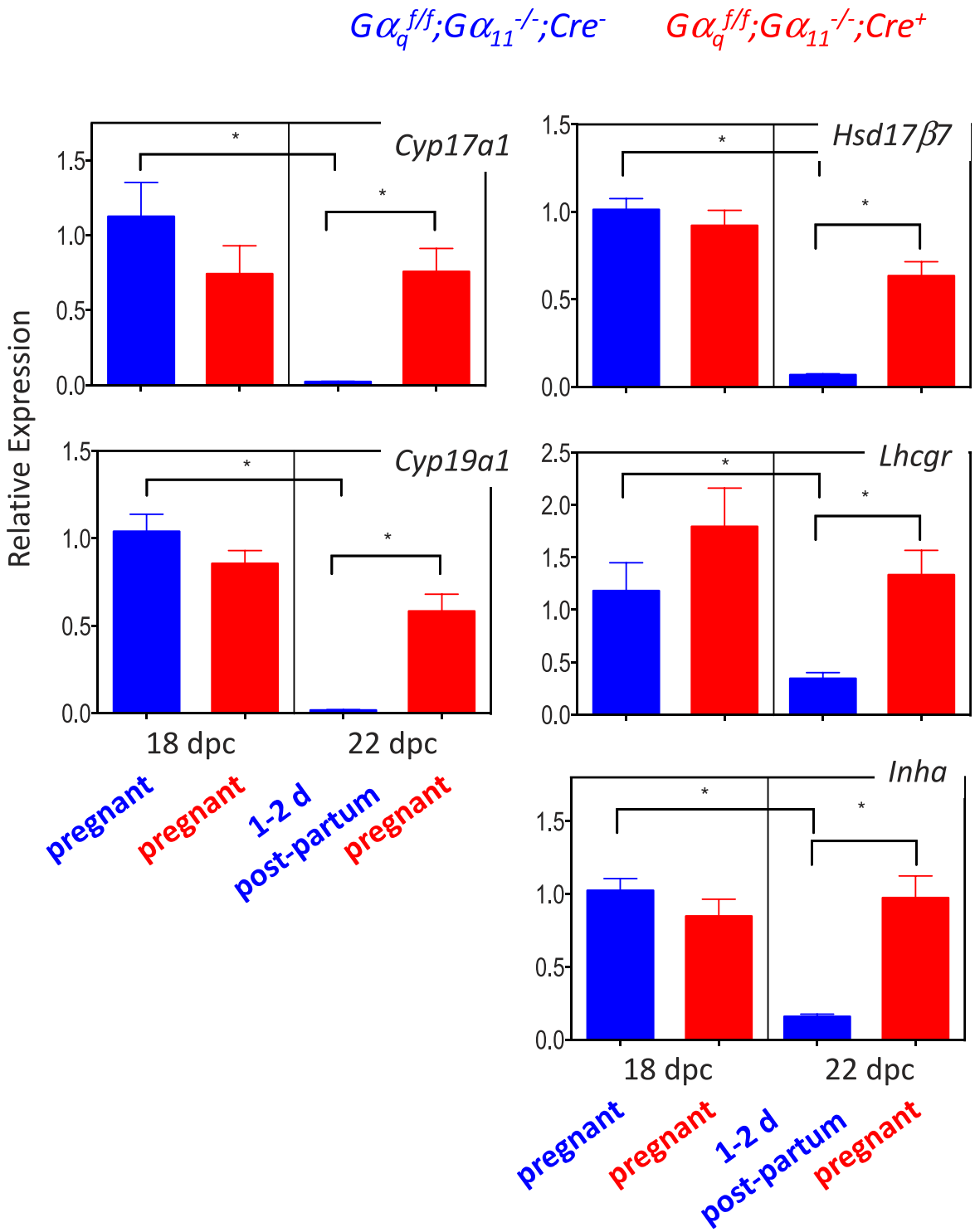

FIG. 5. Quantitative PCR validation of the changes in expression of the genes known to be involved in reproductive functions at the end of gestation in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice. For each gene, data are expressed relative to the gene's expression in the ovaries of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice on 18 dpc (blue bar on the left panel of each graph). Each bar is the mean ± SEM of 8–10 different animals analyzed on the indicated days. Statistically significant differences ($P < 0.05$) were calculated using ANOVA and are shown by the brackets and asterisks. On 18 dpc $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are pregnant. On 22 dpc the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are still pregnant but the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice are 1–2 days postpartum.

contractions and parturition to begin (reviewed in Davis and Rueda [25], Ratajczak and Muglia [39], Stocco et al. [40], and Henkes et al. [41]). We cannot assume, however, that all actions of PGF2α are mediated by $G_{q/11}$, because the PGF2α receptor also activates $G_{12/13}$ [5]. In addition, luteal $G_{q/11}$ can also be activated by other hormones, such as oxytocin [6, 7] or lysophosphatidic acid [8, 9], and these could be involved in luteolysis and parturition as well.

The results of the microarray analysis reported here show that *Ark1c18* is the ovarian gene that is most robustly up-

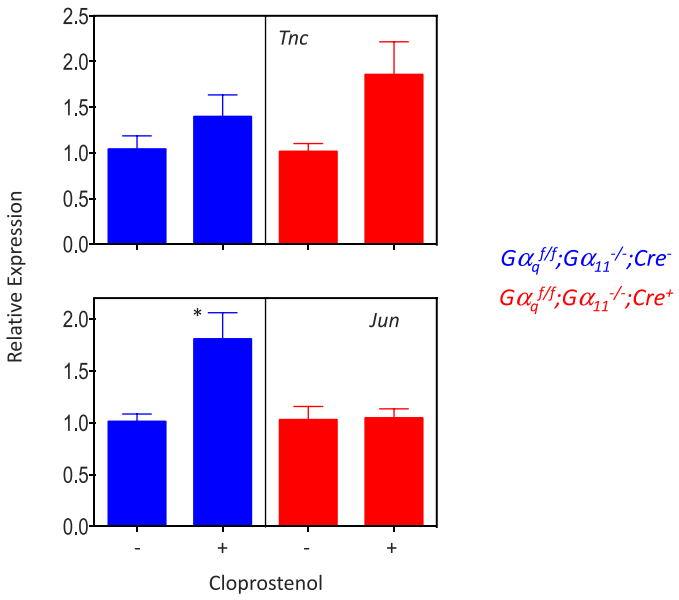

FIG. 6. Effect of cloprostenol on the expression of *Tnc* and *Jun* in the ovaries of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice on 17 dpc. Mice were injected with vehicle or 1 μg of cloprostenol on 17 dpc and killed 24 h later. For each gene, data are expressed relative to the gene's expression in the ovaries of mice of the same genotype injected with vehicle only. Each bar is the mean ± SEM of five different animals. Statistically significant differences ($P < 0.05$) were calculated only by comparing expression in each genotype (*t*-test) and are shown by asterisks.

regulated at the end of gestation in a $G\alpha_{q/11}$-dependent fashion. Because we recently showed that the increased expression of ovarian *Akr1c18* provoked by activation of the PGF2α receptor or observed at the end of gestation is dependent on $G\alpha_{q/11}$ [4], the finding of *Akr1c18* as the ovarian gene that is most robustly up-regulated in our microarray is a reassuring finding that serves as a positive control. The data presented here and in a previous publication [4] also show that the up-regulation of this gene is greatly reduced in magnitude but not abolished in the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice. Nevertheless, this residual up-regulation detected in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice does not appear to be physiologically significant because these mice experience a delay or failure of parturition [4]. When grouped by the magnitude of the difference in expression, the other up-regulated genes code for proteins involved in a variety of cellular functions, including components of the extracellular matrix (*Tnc*), control of the cell cycle (*Cdkn2b*), solute transport (*Slc46a3*), and nicotinic receptor signaling (*Chrna1*). When grouped by their presumed involvement in cellular functions, they segregate into some categories, such as focal adhesion, extracellular matrix, endocytosis, and cytokine signaling, that may be involved in the regression of the corpus luteum and other ovarian remodeling that occurs at the end of gestation and the reentry into the estrous cycle. Although the ''metabolic pathway'' KEGG grouping has the largest number of genes and the highest level of statistical significance we chose not to further consider genes belonging to this KEGG grouping because of its broadness. We instead validated the microarray results of the up-regulated genes with *Tnc* and *Jun*,

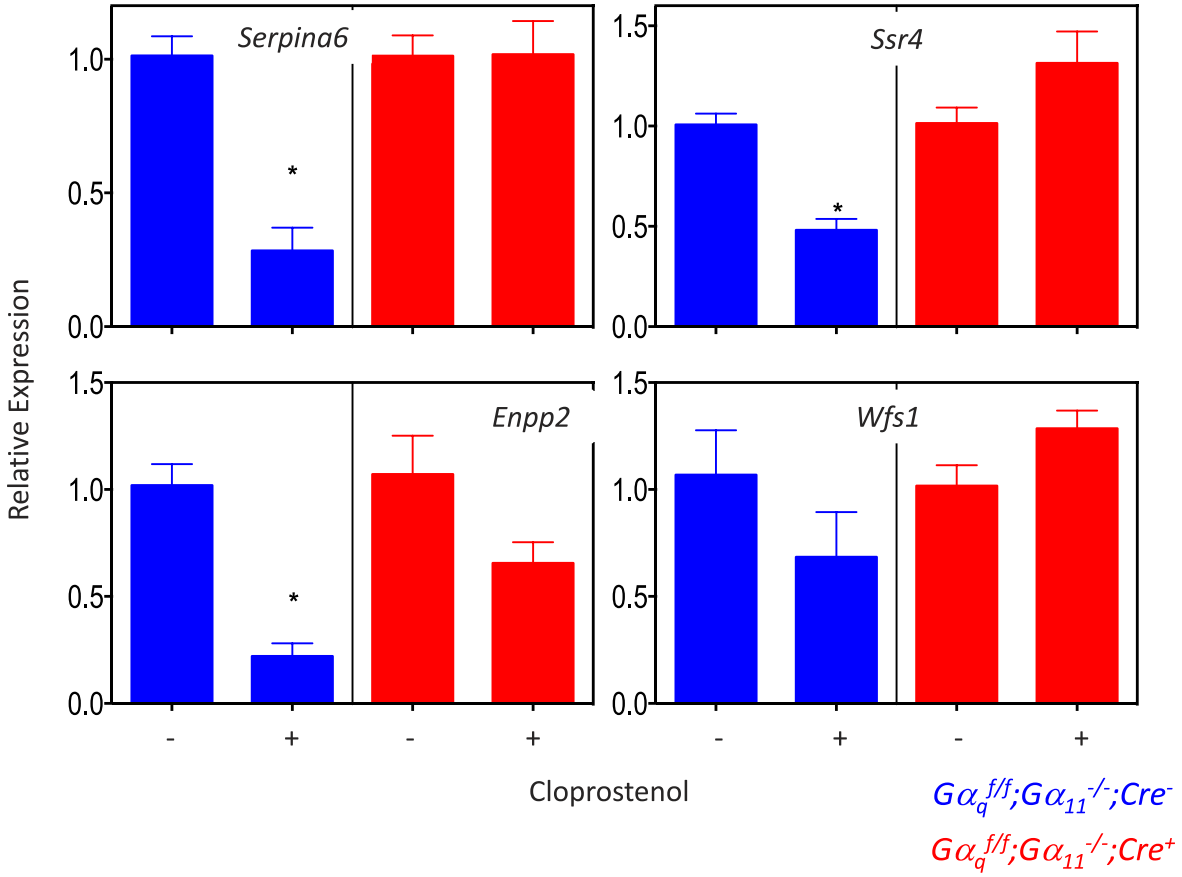

FIG. 7. Effect of cloprostenol on the expression of *Serpina6*, *Enpp2*, *Wfs1*, and *Ssr4* in the ovaries of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice on 17 dpc. Mice were injected with vehicle or 1 μg of cloprostenol on 17 dpc and killed 24 h later. For each gene, data are expressed relative to the gene's expression in the ovaries of mice of the same genotype injected with vehicle only. Each bar is the mean ± SEM of 5–10 different animals. Statistically significant differences ($P < 0.05$) were calculated only by comparing expression in each genotype (*t*-test) and are shown by asterisks.

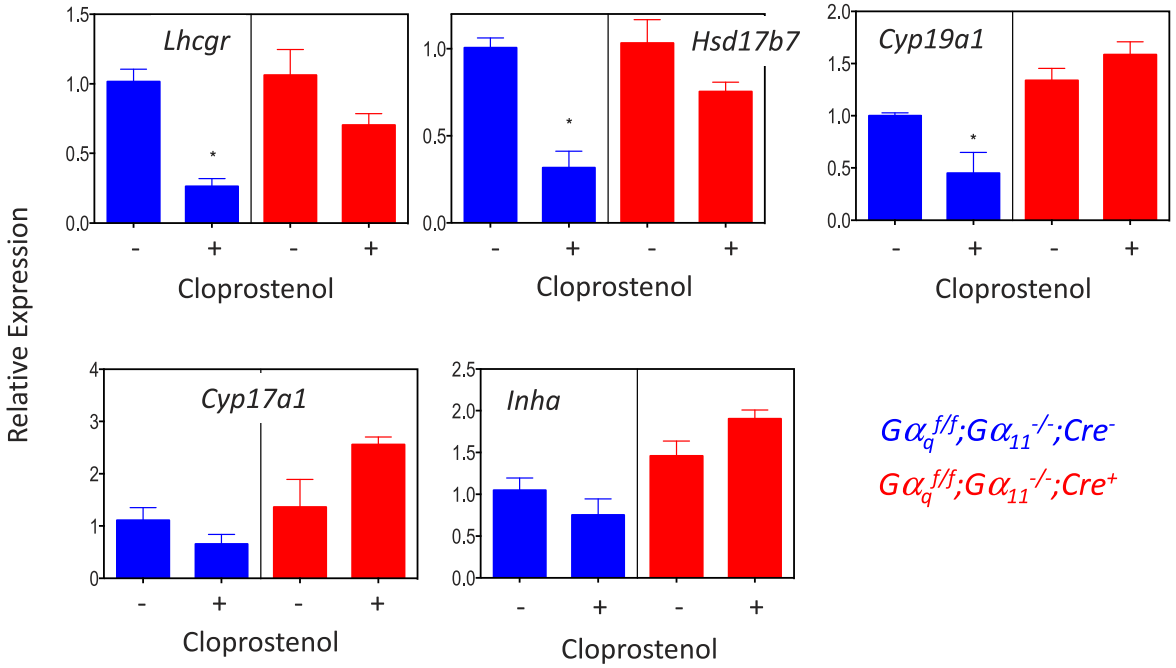

FIG. 8. Effect of cloprostenol on the expression of the genes known to be involved in reproductive functions in the ovaries of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice on 17 dpc. Mice were injected with vehicle or 1 μg of cloprostenol on 17 dpc and killed 24 h later. For each gene, data are expressed relative to the gene's expression in the ovaries of mice of the same genotype injected with vehicle only. Each bar is the mean ± SEM of 5–10 different animals. Statistically significant differences ($P < 0.05$) were calculated only by comparing expression in each genotype (*t*-test) and are shown by asterisks.

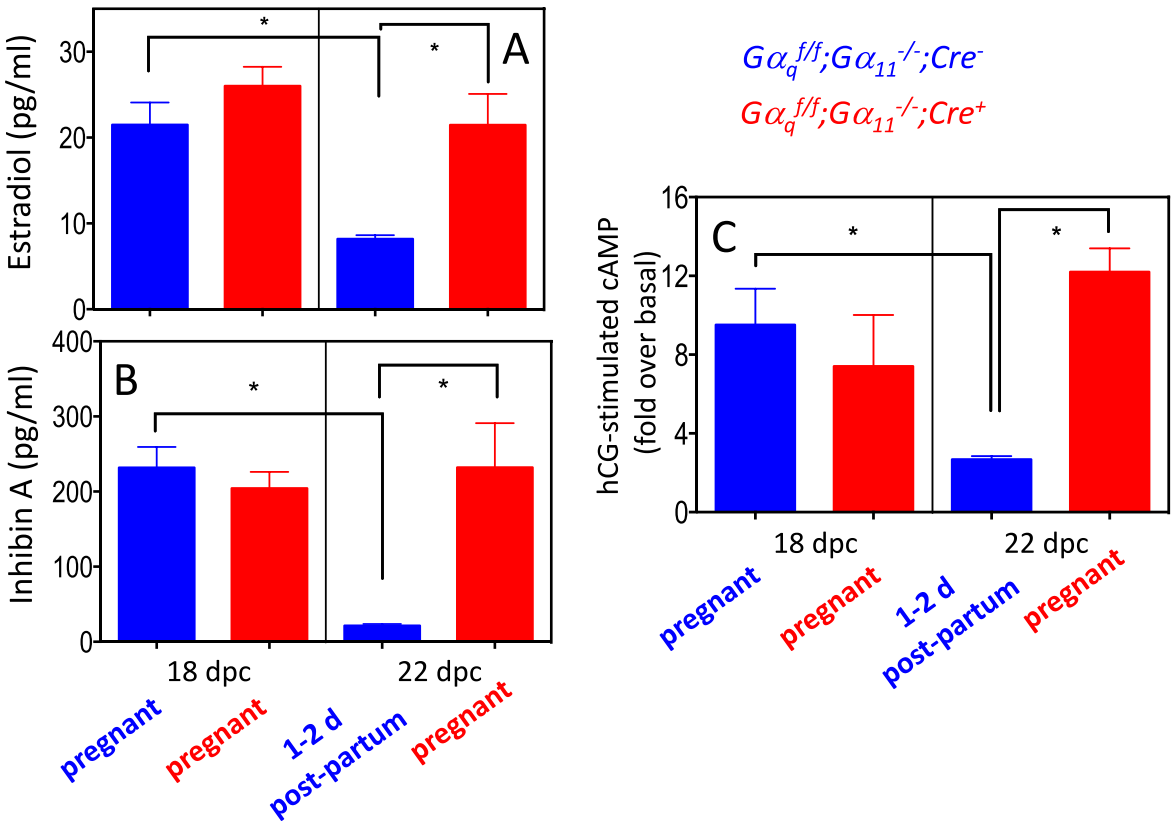

FIG. 9. Physiological correlates of the changes in ovarian reproductive gene expression at the end of gestation in $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice. For **A** and **B**, each bar shows the mean ± SEM of five to eight different animals analyzed on the indicated days. **C**) The effects of hCG on cAMP accumulation in freshly isolated luteal cells (see *Materials and Methods* for details). These data are presented as fold over basal level for ease of comparison. The basal levels of cAMP among the four groups shown varied between 30 and 50 pmol/mg protein. Each bar shows the mean ± SEM of three to four different animals analyzed on the indicated days. Statistically significant differences ($P < 0.05$) were calculated using ANOVA and are shown by the brackets and asterisks. On 18 dpc $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ and $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are pregnant. On 22 dpc the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice are still pregnant but the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice are 1–2 days postpartum.

two up-regulated genes placed in the focal adhesion KEGG pathway. *Tnc* codes for tenascin C (Tn-C), a nonstructural extracellular matrix protein that is highly up-regulated during active tissue remodeling [42]. Tn-C is expressed in several ovarian compartments, including degenerating luteal cells [43–45]. *Jun* was also placed in this KEGG pathway, but because it is a component of the AP-1 transcription factor [46], it can modulate the expression of many genes and participate in many pathways. *Jun* is present in the porcine corpus luteum, and under some conditions it can be induced by PGF2α [47].

Serpina6 and *Enpp2* are the genes that exhibit the largest $G\alpha_{q/11}$-dependent decrease in expression at the end of gestation. The finding that these two genes are expressed in the ovary and down-regulated at the end of gestation is novel and could have potential implications in reproductive functions. *Serpina6* codes for corticosteroid-binding globulin (CBG), a circulating protein that binds cortisol and progesterone [48]. In the rat, the liver is the main source of circulating CBG, and its expression can be stimulated with estrogens [49, 50]. Circulating CBG levels increase during the second and third trimesters in pregnant women and influence progesterone levels in the blood and at the maternal-fetal interface [51, 52]. Our data show that *Serpina6* is expressed in the pregnant mouse ovary and that it declines at the end of gestation in a $G\alpha_{q/11}$-dependent fashion. The functions of ovarian *Serpina6* and its possible contribution to circulating CBG remain to be determined, however. *Enpp2* codes for autotaxin [53–55], an extracellular enzyme that synthesizes lysophosphatidic acid (LPA). Lysophosphatidic acid is an extracellular phospholipid that activates many cellular functions by engaging one or more of the six known LPA receptors [8, 9], but its role in reproduction in general and ovarian physiology in particular are poorly understood [9]. The human ovary was initially reported as a site of high *Enpp2* expression [56], but this was not confirmed by the expression data available on public databases [57]. Autotaxin expression was shown in the rat corpus luteum but not in developing or mature follicles [58]. In addition, LPA is present in very high concentrations in human follicular fluid isolated from women undergoing in vitro fertilization, and circulating LPA as well as circulating autotaxin increase during human pregnancy [59, 60]. Lastly, several LPA receptor subtypes are expressed in the ovary [9, 58], and addition of LPA to luteal cells impacts steroid and interleukin biosynthesis [61, 62]. When considered together, these data suggest that ovarian-derived LPA is an autocrine/paracrine regulator of ovarian functions. This latter possibility is currently being investigated.

When grouped by their presumed involvement in cellular functions (Table 4), the genes that are down-regulated at the end of gestation in a $G\alpha_{q/11}$-dependent fashion segregate into several KEGG pathways, including three—''protein processing in endoplasmic reticulum,'' ''*N*-glycan biosynthesis,'' and ''protein export''—that are involved in protein synthesis, processing, and glycosylation. We validated the microarray results of the down-regulated genes with *Wfs1 and Ssr4*, two down-regulated genes placed in the ''protein processing in endoplasmic reticulum'' KEGG pathway, the down-regulated pathway with the highest level of statistical significance. *Wfs1* codes for an endoplasmic reticulum protein called WFS1 that is involved in calcium homeostasis and the unfolded protein response. In humans, mutations of the *WFS1* gene result in a disorder (called Wolfram syndrome) characterized by neurodegeneration and diabetes mellitus [63]. *Ssr4* codes for SSR4, one of the four signal sequence receptor proteins that are part of a complex of membrane proteins needed for *N*-glycosylation [64] and to translocate secreted proteins across the endoplasmic reticulum membrane [65]. There is no available information about the expression or possible functions of these two genes in the ovary, but because of the secretory nature of luteal cells [66], the down-regulation of these ovarian genes could also be involved in the transition that occurs as the mice resume the estrous cycle after delivery.

The group of down-regulated genes in the ''steroid hormone biosynthesis'' KEGG pathway is also of interest because it contains the three enzymes required to convert progesterone to estradiol (*Cyp17a1*, *Hsd17b7*, and *Cyp19a1*). These three genes, as well as two other ovarian genes known to be involved in reproductive functions (*Lhcgr* and *Inha*), are also among the genes with the largest $G\alpha_{q/11}$-dependent decrease in expression at the end of gestation. Thus, we also validated the microarray results with these five additional genes. Two of these (*Cyp19a1* and *Hsd17b7*) were previously shown to decrease at the end of gestation [30, 67], but our finding that they are dependent on $G\alpha_{q/11}$ is new. The changes in expression of these five reproductive genes have clear physiological correlates. The decrease in *Lhcgr* reduces the responsiveness of luteal cells to hCG, the decrease in *Inha* results in a reduction in the circulating levels of Inhibin A, and the decrease in the three steroidogenic enzymes is associated with a decrease in circulating estradiol levels. We hypothesize that the $G\alpha_{q/11}$-dependent decrease in expression of *Lhcgr*, *Cyp17a1*, *Cyp19a1*, and *Hsd17b7*, and the increase in expression of *Akr1c18* coordinate the drop in circulating progesterone and estradiol that occurs at the end of gestation [4, 25–29, 39–41]. The decrease in *Lhcgr* reduces the stimulus for progesterone synthesis, and the increase in *Akr1c18* enhances progesterone metabolism. Because the magnitude of progesterone production is several orders of magnitude higher than that of estradiol (ng/ml levels of circulating progesterone and pg/ml levels for circulating estradiol), a decline in progesterone production alone (such as that induced by the changes in *Lhcgr* and *Akr1c18*) would have to be very large to translate into a significant drop in estradiol production. Thus, the additional decrease in expression of *Cyp17a1*, *Cyp19a1*, and *Hsd17b7* is likely to contribute to a reduction in estradiol synthesis even if the substrate (progesterone) was still relatively high.

The decreased expression of *Inha* and circulating Inhibin A predicts that follicle-stimulating hormone (FSH) levels will rebound at the end of gestation in the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^-$ mice but remain low in the $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice. We attempted to test this hypothesis by measuring FSH in both groups of mice on 22 dpc, but most of the samples collected had undetectable levels of FSH.

The failure of parturition and lack of progesterone withdrawal observed in the *Ptgfr* null mice shows that activation of the luteal PGF2α receptor is the trigger for parturition in mice (reviewed in Davis and Rueda [25], Ratajczak and Muglia [39], Stocco et al. [40], and Henkes et al. [41]). Because we have recently shown that the phenotype of $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice is very similar to that of the *Ptgfr* null mice, and because luteal cells derived from $G\alpha_q^{f/f};G\alpha_{11}^{-/-};Cre^+$ mice fail to respond to PGF2α with an increase in *Akr1c18* expression, we concluded that the effects of the luteal PGF2α receptor on the induction of *Akr1c18* are mediated by the activation of $G_{q/11}$ [4]. These results, together with the $G\alpha_{q/11}$ dependency of the changes in gene expression that occur at the end of gestation, suggest that they are induced by PGF2α. Indeed, (in addition to *Akr1c18*) some of the genes identified here have already been shown to be targets of PGF2α. The decreased expression of ovarian *Hsd17b7* and *Cyp19a1* that occurs at the end of gestation was shown to be blunted in *Ptgfr* null mice [67], but the data reported here show for the first time that this effect is dependent on the granulosa/luteal $G\alpha_{q/11}$. Also,

a previous study has shown that administration of PGF2α to pseudopregnant rats decreases the binding of ^{125}I-hCG to the corpus luteum and the ability of hCG to stimulate progesterone synthesis [68]. Our findings that *Lhcgr* declines at the end of gestation in a $G\alpha_{q/11}$-dependent fashion are also new. When we tested the effects of a PGF2α agonist on the expression of 11 of the $G\alpha_{q/11}$-dependent genes characterized here, we found that only 7 of them (*Jun*, *Serpina6*, *Ssr4*, *Enpp2*, *Lhcgr*, *Hsd17b7*, and *Cyp19a1*) are regulated by PGF2α, whereas the other 4 (*Tnc*, *Wfs1*, *Cyp17a1*, and *Inha*) are not. It is possible, however, that some of the PGF2-insensitive mRNAs have a relatively long half-life, and therefore changes in expression may not be detectable within 24 h. In contrast, the microarray and the qPCR experiments done at the end of gestation were done by comparing animals on 18 and 22 dpc (a 96-h difference). Another possibility is that the expression of these genes is dependent on the actions of endocrine or paracrine/autocrine hormones that also activate luteal $G_{q/11}$ (such as oxytocin, see Blanks and Thornton [6] and Mitchell et al. [7]) or LPA [8, 9]. When engaged by oxytocin, the oxytocin receptor is known to activate $G_{q/11}$ [6, 7], and oxytocin has been shown to induce progesterone withdrawal and parturition in *Ptgr* null mice [69]. The actions of LPA in the corpus luteum need to be characterized, but some of the LPA receptors are known to activate $G_{q/11}$ [8, 9]. The changes in the expression of $G\alpha_{q/11}$-dependent but PGF2α-independent genes may also not be a direct effect of the activation of $G_{q/11.}$ It may be secondary to the decrease in estradiol levels that occurs as a consequence of $G_{q/11}$ activation. Lastly, the dependency of luteal *Cyp17a1* expression on $G\alpha_{q/11}$ is also curious because *Cyp17a1* is expressed in theca cells, not granulosa cells [70], and the deletion of $G\alpha_{q/11}$ took place initially in granulosa cells [3, 4]. We therefore hypothesize that the regulation of luteal *Cyp17a1* expression is due to $G\alpha_{q/11}$-dependent changes in a granulosa/luteal cell-specific product that acts on theca/luteal cells to inhibit *Cyp17a1* expression.

In summary, the results presented here have identified 138 and 150 ovarian genes that are up-regulated or down-regulated, respectively, at the end of gestation in a $G\alpha_{q/11}$-dependent fashion. Genes that participate in the structure/function of the extracellular matrix are among those that are up-regulated, whereas some of the down-regulated genes participate in the processing and transport of proteins in the endoplasmic reticulum. Several ovarian genes known to be involved in reproductive functions and two novel ovarian genes that may participate in reproductive functions were also down-regulated in a $G\alpha_{q/11}$-dependent fashion. These changes in ovarian gene expression are likely to be involved in the transition that occurs at the end of gestation and in the reentry into the estrous cycle. PGF2α, acting thought the luteal prostaglandin F2α receptor, a $G_{q/11}$-coupled receptor, is a prominent regulator of luteolysis, but not all of the genes that are regulated at the end of gestation in a $G\alpha_{q/11}$-dependent fashion are targets of PGF2α

ACKNOWLEDGMENT

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