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Progesterone Inhibits Uterine Gland Development in the Neonatal Mouse Uterus[1]

Justyna Filant,[3] Huaijun Zhou,[4] and Thomas E. Spencer[2,3]

[3]*Center for Reproductive Biology, Department of Animal Sciences, Washington State University, Pullman, Washington*
[4]*Department of Poultry Science, Texas A&M University, College Station, Texas*

ABSTRACT

Uterine glands and their secretions are required for conceptus (embryo/fetus and associated placenta) survival and development. In most mammals, uterine gland morphogenesis or adenogenesis is a uniquely postnatal event; however, little is known about the mechanisms governing the developmental event. In sheep, progestin treatment of neonatal ewes permanently ablated differentiation of the endometrial glands. Similarly, progesterone (P4) inhibits adenogenesis in neonatal mouse uterus. Thus, P4 can be used as a tool to discover mechanisms regulating endometrial adenogenesis. Female pups were treated with sesame vehicle alone as a control or P4 from Postnatal Day 2 (PD 2) to PD 10, and reproductive tracts were examined on PD 5, 10, or 20. Endometrial glands were fully developed in control mice by PD 20 but not in P4-treated mice. All other uterine cell types appeared normal. Treatment with P4 stimulated proliferation of the stroma but suppressed proliferation of the luminal epithelium. Microarray analysis revealed that expression of genes were reduced (*Car2*, *Fgf7*, *Fgfr2*, *Foxa2*, *Fzd10*, *Met*, *Mmp7*, *Msx1*, *Msx2*, *Wnt4*, *Wnt7a*, *Wnt16*) and increased (*Hgf*, *Ihh*, *Wnt11*) by P4 in the neonatal uterus. These results support the idea that P4 inhibits endometrial adenogenesis in the developing neonatal uterus by altering expression of morphoregulatory genes and consequently disrupting normal patterns of cell proliferation and development.

early development, mechanisms of hormone action., rodents (guinea pigs, mice, rats, voles), steroid hormones, steroid hormone receptors, uterus

INTRODUCTION

Development of the uterus is initiated in the fetus with formation, patterning, and fusion of the Müllerian ducts [1, 2]. However, establishment of tissue-specific histoarchitecture is completed only after birth in laboratory rodents and domestic animals [3–5]. At birth, the mouse uterus lacks endometrial glands and consists of a simple luminal epithelium (LE) supported by undifferentiated mesenchyme [6]. During the next 3 days of postnatal life, the mesenchyme segregates into radially oriented endometrial stroma and inner circular and prospective outer longitudinal myometrial layers. Between birth (Postnatal Day [PD] 0) and PD 6, glandular epithelial (GE) cells differentiate and bud from the LE [7]. By PD 12, GE buds extend from the LE into the surrounding endometrial stroma, and the inner and outer layers of the myometrium are fully organized [6]. By PD 15, the histoarchitecture of the uterus resembles that of the adult [5, 8].

Development of the endometrial glands or adenogenesis is a critical event in uterine morphogenesis because it determines, in part, the embryotrophic potential of the adult uterus [4, 9]. The endometrial glands of the uterus produce substances that are critical for conceptus (embryo/fetus and associated placenta) survival, development, and implantation [5, 10, 11]. For example, leukemia inhibitory factor (Lif) is produced and secreted exclusively by endometrial glands of the mouse uterus [12], and *Lif* null mice are infertile due to a failure of blastocyst implantation [13]. Studies of postnatal uterine development, as well as the regenerating endometrium of the adult, implicate cell proliferation and epithelium-stroma interactions in uterine development and endometrial adenogenesis [3, 5, 14, 15]. Communication between the epithelium and stroma appears to be mediated by *Wnt* and *Hox* genes, intrinsic growth factor systems, and changes in the composition and distribution of the extracellular matrix [3, 16, 17]. Mutant mouse models have been used to identify a number of genes involved in uterine gland development including *Foxa2*, *Hoxa10*, *Hoxa11*, *Wnt4*, *Wnt5a*, and *Wnt7a* [1–5, 5, 18–22].

One approach to discover novel regulatory genes and pathways is to disrupt uterine morphogenesis by using steroid hormones, given that the initial stages of postnatal uterine development are ovary- and steroid-independent [5, 23]. For example, treatment of newborn ewe lambs with progestin from birth to PD 56 permanently ablated endometrial gland development, resulting in an adult uterine gland knockout ewe that exhibited recurrent early pregnancy loss due to defects in peri-implantation conceptus survival and growth [24]. In neonatal mice, progesterone receptor (PGR) protein can be detected in the uterine LE by PD 3 and in the stroma by PD 6 [25, 26]. Recently, exposure of neonatal mice to P4 from birth to PD 5 was found to inhibit but not permanently ablate endometrial adenogenesis [27]. However, exposure of neonatal mice to P4 from PD 3 to PD 10 permanently ablated endometrial adenogenesis [28]. The objective here was to use neonatal P4 exposure as a tool to discover new cellular and molecular mechanisms governing postnatal endometrial adenogenesis and uterine development in mice.

MATERIALS AND METHODS

Animals and Tissue Collection

All animal procedures were approved by the Institutional Animal Care and Use Committee and were conducted according to Guide for the Care and Use of Laboratory Animals. In Study One, a total of 24 litters of C57BL/6J mice were randomly assigned to one of two treatment groups at birth (defined as PD 0). All female pups within a litter received daily subcutaneous injections of sesame oil vehicle alone as a control or P4 (50 μg/g of body weight) dissolved in sesame oil from PD 2 to 10. Female pups were necropsied on PD 5, 10, or 20 ($n \geq 4$ litters per PD and treatment). One uterine horn was fixed in 4% paraformaldehyde in PBS (pH 7.2) at room temperature for 24 h and embedded in paraffin for histology. The remaining uterine horn was snap frozen in liquid nitrogen and stored at −80°C. In Study Two, female pups were treated with

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[2]Correspondence: E-mail: thomas.spencer@wsu.edu

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vehicle alone or with P4 from PD 2 to 10 as described above, and uteri were collected at 8 wk of age (n = 6 females/treatment) for histological analysis.

Transcriptional Profiling Using Agilent Microarrays

Total RNA was extracted from frozen uteri (n = 4 PD-10 pups per treatment) by using a Qiagen RNeasy mini-kit (Valencia, CA) and then treated with DNase I (Qiagen). The integrity and quantity of total RNA were determined using an Agilent Bioanalyzer 2100 Lab-on-chip system (Agilent Technologies, Palo Alto, CA). Total RNA (400 ng) was reverse transcribed to cDNA, during which a T7 sequence was introduced into the cDNA. T7 RNA polymerase-driven RNA synthesis was used for preparation and labeling of RNA with Cy3 (or Cy5) dye. Fluorescent cRNA probes were purified using a Qiagen RNeasy mini-kit, and equal amounts (825 ng) of Cy3- and Cy5-labeled cRNA probes were hybridized to a 44K whole-mouse genome Agilent array (catalog no. G2519F-014868). The hybridized slides were washed using Agilent reagents and then scanned using a Genepix 4100A scanner (Molecular Devices Corp., Sunnyvale, CA) with a tolerance of saturation setting of 0.005%. For each channel, the median of the signal intensity and local background values were used. A locally weighted linear regression normalization was applied to remove signal intensity-dependent dye bias for each array by using R software [29]. The Student *t*-test was used to identify differentially expressed genes, and a *P* value of 0.01 or less was considered significant.

Database for Annotation, Visualization, and Integrated Discovery Analysis

Database for annotation, visualization, and integrated discovery (DAVID) version 6.7 software (http://david.abcc.ncifcrf.gov/) enables using microarray gene lists to generate specific functional annotations of biological processes affected by treatment in microarray experiments [30, 31] and was used to annotate biological themes in the uterus affected by P4 treatment. All differentially expressed genes identified as both significantly ($P < 0.01$) and numerically (1.5-fold change) different between control and P4-treated mice were used in the DAVID analysis.

Semi-Quantitative Real-Time RT-PCR Analysis

Semi-quantitative real-time RT-PCR (qPCR) analysis was conducted using total RNA extracted from the uteri (n = 4 pups per PD and treatment) and by methods described by our laboratory [20]. The primers used for PCR analysis are provided in Supplemental Table S1 (all Supplemental Data are available online at www.biolreprod.org). Mouse *Rpl13a* was used as a reference gene. *Foxa2* was determined by TaqMan analysis with TaqMan gene expression Master Mix (Applied Biosystems, Carlsbad, CA) and normalized against *Rn18s*. Real-time probes and primers for *Foxa2* and *Rn18s* were purchased from Applied Biosystems.

Immunohistochemistry

Paraffin-embedded uteri (n = 4 pups per PD and treatment) were sectioned at a thickness of 5 μm and mounted on slides, deparaffinized, and rehydrated in a graded alcohol series. Sections were subjected to antigen retrieval using boiling citrate buffer, preincubated with 10% normal goat serum in PBS (pH 7.5), and then incubated with primary antibody or nonimmune normal immunoglobulin G (IgG; Sigma-Aldrich) at the same final concentration as the negative control. Primary antibodies were diluted in 1% BSA in PBS (pH 7.5) as follows: anti-MKI67 at 1:750 dilution (catalog no. ab66155; Abcam, Cambridge, MA) and anti-FOXA2 at 1:7000 dilution (catalog no. WRAB-FOXA2; Seven Hills Bioreagents, Cincinnati, OH). Sections were then washed in PBS and incubated with biotinylated secondary antibody (5 μg/ml; Vector Laboratories, Burlingame, CA). Immunoreactive protein was visualized using a Vectasin ABC kit (Vector Laboratories) and diaminobenzidine tetrahydrochloride as the chromagen. Some sections were counterstained with hematoxylin before affixing coverslips with Permount (Fisher Scientific, Fairlawn, NJ).

Statistical Analyses

All quantitative data was subjected to least-squares ANOVA using general linear model procedures of Statistical Analysis System (SAS Institute Inc., Cary, NC). In all analyses, error terms used in tests of significance were identified according to the expectation of the mean squares for error. For analysis of real-time PCR data, the C_t values of the target mRNA were analyzed for effects of day, treatment (control or P4), and their interaction with the *Rpl13a* or *Rn18s* values used as a covariate. Significance ($P < 0.05$) was determined by probability differences of least-squares means.

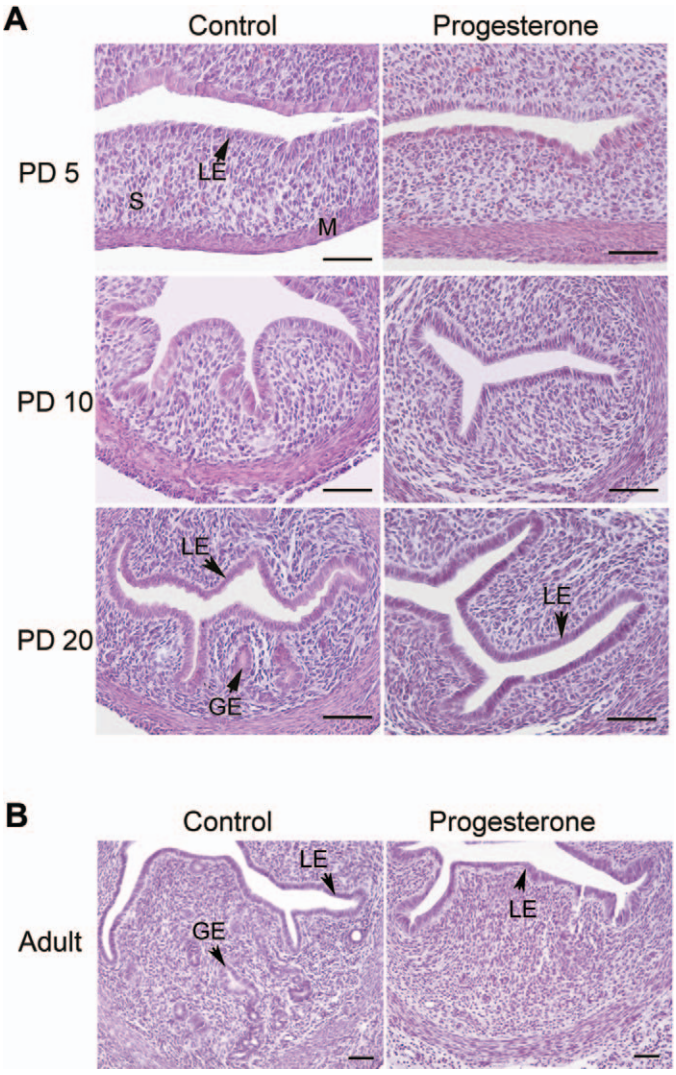

FIG. 1. Neonatal progesterone treatment inhibits endometrial gland development in the mouse uterus. Mice were treated with sesame oil vehicle as a control or P4 in vehicle from PD 2 to 10 after birth. Histological examination of the uteri collected on PD 5, 10, and 20 and at 8 wk of age from control mice (left) and P4-treated mice (right). Sections of uteri were stained with hematoxylin and eosin. Note the absence of endometrial glands in the uteri of P4-treated mice as compared to control mice. LE, luminal epithelium; GE, glandular epithelium; M, myometrium; S, stroma. Bar = 50 μm.

RESULTS

Neonatal Progesterone Exposure Ablates Endometrial Gland Development

The morphology of the uterus was not different between control and P4-treated mice on PD 5 (Fig. 1). Uteri contained a low columnar LE supported by stroma and myometrium. In control mice, endometrial glands were absent on PD 5, whereas budding and differentiating GE were observed in uteri on PD 10. By PD 20, uteri of control mice contained distinct glands in the stroma. Treatment of female mice with P4 from PD 2 to 10 inhibited endometrial adenogenesis, because no endometrial glands were observed in the uteri of mice treated with P4 (Fig. 1A). All other cell types appeared histologically normal in the

uteri from P4-treated mice. Interestingly, the uteri of 8-wk old adult females treated with P4 from PD 2 to 10 had no uterine glands but did contain histologically normal LE, stroma, and myometrium (Fig. 1B).

Forkhead box A2 (FOXA2) protein was evaluated because it is a primary regulator of gland development in mice [19, 20]. As illustrated in Figure 2A, *Foxa2* mRNA increased substantially in the uteri of control mice between PD 5 and 20. In contrast, no increase in *Foxa2* mRNA was observed in uteri of P4-treated mice. On PD 5 and 10, immunoreactive FOXA2 protein was detected in the cytoplasm of LE and some stromal cells in uteri of both control and P4-treated mice (Fig. 2B). The FOXA2 protein was in the nuclei of some stromal cells on PD 5. In contrast, abundant FOXA2 protein was observed in the nuclei of GE cells of uteri from control mice on PD 20.

Progesterone Alters Uterine Cell Proliferation

To investigate P4 effects on cell proliferation, MKI67, a cellular marker of proliferation [32], was evaluated in the uterus (Fig. 3). In control mice, the number of MKI67-positive cells in the stroma and myometrium was maximal on PD 5 and decreased thereafter. The number of MKI67-positive cells in the LE was high on PD 5 and 10. No MKI67-positive cells were observed in the LE of control mice on PD 20, and only a few stromal and myometrial cells were positive. Treatment with P4 increased the number MKI67-positive cells in the stroma on PD 5, 10, and 20 but decreased the number of MKI67-positive cells in the LE on PD 5 and 10 (Fig. 3).

Progesterone Affects Gene Expression in the Neonatal Uterus

Microarray analysis found that 497 and 564 Agilent probe sets (each representing a gene transcript) were increased or decreased ($P < 0.01$ and $\geq$1.5-fold change), respectively, in the P4-treated uteri compared to control PD 10 uteri (Supplemental Tables S2 and S3). DAVID analysis revealed that P4 treatment increased expression of genes associated with extracellular matrix (ECM)-receptor interactions, focal adhesion, and transforming growth factor (TGF)-beta signaling pathways (Table 1). In contrast, P4 treatment decreased expression of genes associated with cell cycle, DNA replication, and cell adhesion pathways (Table 2).

A selected number of differentially expressed genes were validated by qPCR with a focus on genes implicated in uterine development, including WNTs and their Frizzled (FZD) receptors; WNT signaling inhibitors; HOX transcription factors; growth factors and their receptors; and metalloproteinases and regulators of their activity. As presented in Figure 4, neonatal P4 treatment increased (day x treatment; $P < 0.05$) expression of both *Fst* and *Ihh* on PD 10, which are well known P4-stimulated genes in the mouse uterus [33]. Treatment with P4 decreased ($P < 0.05$) expression of *Wnt4*, *Wnt7a*, and *Wnt16*. In contrast, *Wnt11* was increased by P4 on PD 10 (day x treatment, $P < 0.05$). Neonatal exposure to P4 did not affect ($P > 0.10$) *Wnt5a*. *Fzd6* and *Fzd10* are the most abundantly expressed WNT receptors in the neonatal mouse uterus [34]. Treatment with P4 decreased expression of *Fzd6* on PD 5 and reduced *Fzd10* expression on PD 5 and 10 (day x treatment, $P < 0.05$). Expression of the WNT signaling inhibitor *Sfrp4* was increased on PD 10 and 20 by P4 (day x treatment; $P < 0.05$).

Two msh homolog homeobox genes (*Msx1* and *Msx2*) were expressed in the mouse uterus [35, 36]. As illustrated in

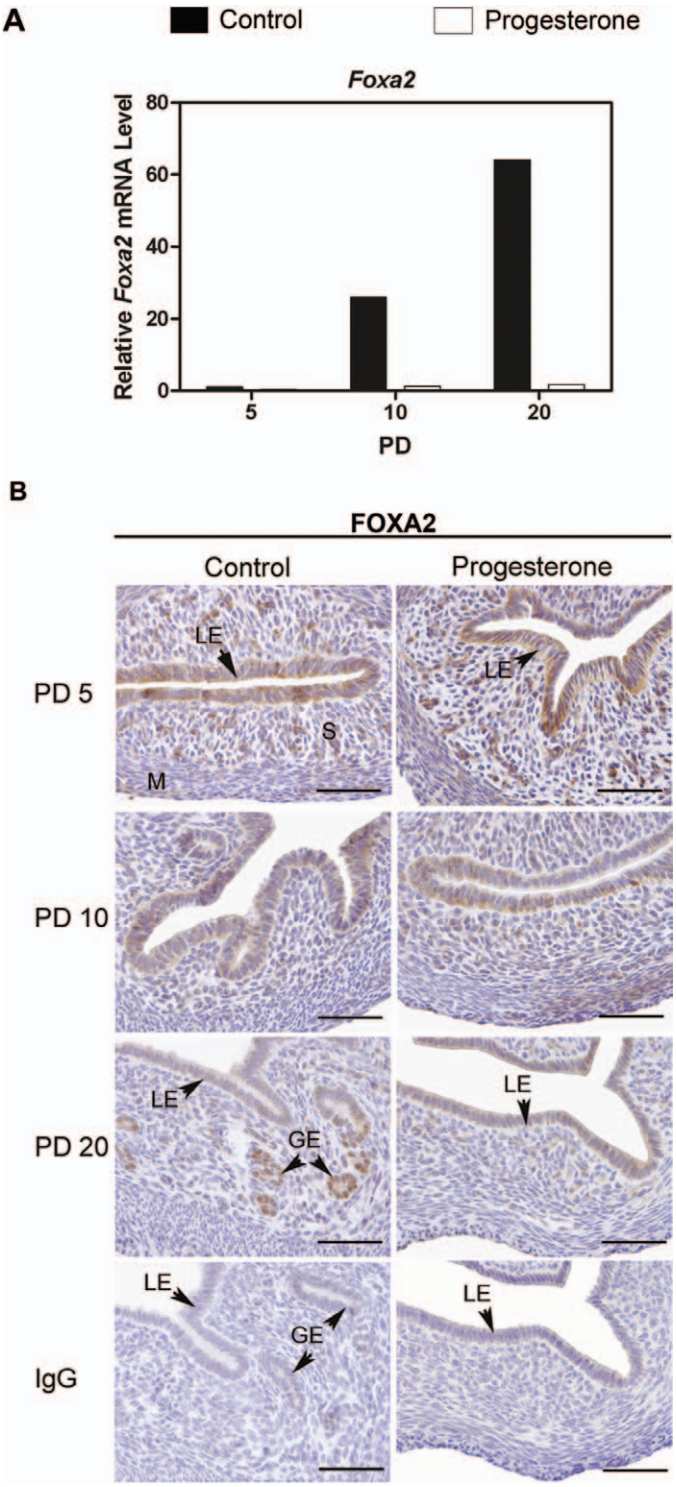

FIG. 2. Expression and localization of FOXA2 in the uterus of control and progesterone-treated mice. Mice were treated with sesame oil vehicle as a control or P4 in vehicle from postnatal day (PD) 2 to 10 after birth. **A**) The relative mRNA levels of *Foxa2* was measured in the uterus of control and P4-treated mice by real-time RT-PCR. Data are presented as fold change relative to the mRNA level on PD 5 in uteri from control mice. **B**) Immunohistochemical localization of FOXA2 protein in uteri of control and P4-treated mice on PD 5, 10, and 20. Sections were counterstained with hematoxylin after immunolocalization. Note the presence of FOXA2 protein in the nuclei of endometrial glands. LE, luminal epithelium; GE, glandular epithelium; M, myometrium; S, stroma. Bar = 50 μm.

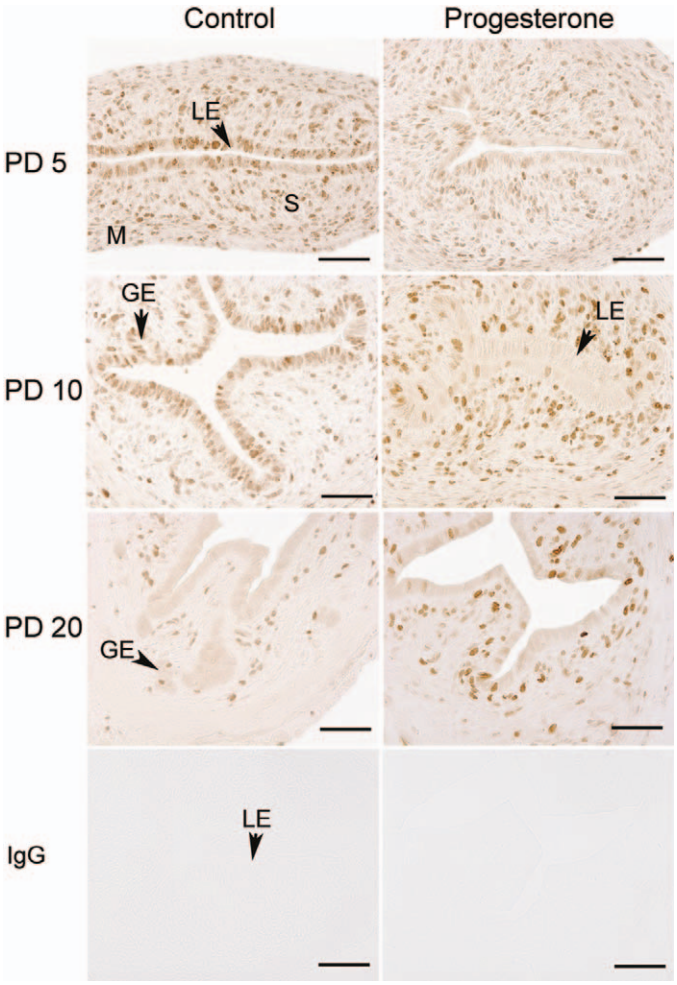

FIG. 3. Immunohistochemical localization of MKI67 in the uteri of control and progesterone-treated mice. Mice were treated with sesame oil vehicle as a control or P4 in vehicle from PD 2 to 10 after birth. Cell proliferation was assessed by immunostaining uteri for MKI67. Sections were not counterstained with hematoxylin after immunolocalization. LE, luminal epithelium; GE, glandular epithelium; M, myometrium; S, stroma. Bar = 50 μm.

Figure 4, *Msx1* expression increased ($P < 0.05$), while *Msx2* expression decreased ($P < 0.05$) between PD 5 and 20 in uteri from control mice. Treatment with P4 decreased ($P < 0.05$) expression of both *Msx1* and *Msx2*.

Metalloproteinases are involved in extracellular matrix remodeling, and *Mmp2*, *Mmp7*, and the metalloproteinase regulators *Car2* and *Adamts1* are expressed in the developing and adult mouse uterus [37, 38]. As illustrated in Figure 5, *Adamts1* and *Mmp2* expression were not affected ($P > 0.10$) by P4 treatment, whereas P4 decreased ($P < 0.05$) *Mmp7* on all PDs and reduced (day x treatment, $P < 0.05$) *Car2* expression on PD 5 and 10.

A number of growth factors and their receptors are implicated in P4 inhibition of uterine adenogenesis in neonatal ewes [24]. As illustrated in Figure 5, expression of *Met* and *Fgfr2* were decreased (day x treatment, $P < 0.05$) by P4 treatment, whereas *Hgf* was increased (day x treatment, $P < 0.05$) on PD 10 by P4 treatment. In contrast, *Fgf7* and *Fgf10* were not affected ($P > 0.10$) by P4 treatment.

DISCUSSION

This study supports the idea that P4 can be used as an effective tool with which to discover new factors and pathways regulating postnatal endometrial adenogenesis and uterine development in the mouse. In the present study, P4 treatment of neonatal C57BL/6J mice altered expression of morpho-regulatory genes and epithelial cell proliferation, leading to a disruption of endometrial adenogenesis in the developing uterus. Histological analysis of adult uterine tissues revealed the absence of endometrial glands in neonatally progestinized mice. Similarly, Cooke et al. [28] recently reported that treatment of C57BL/6J mice with P4 from PD 3 to 10 permanently blocked endometrial adenogenesis and resulted in adult infertility. In contrast, treatment of Swiss Webster mice with P4 from PD 0 to 10 reduced but did not permanently ablate endometrial adenogenesis [27]. In that study, the uteri of neonatally P4-treated Swiss Webster mice had substantially reduced numbers of uterine glands at 3 and 8 wk of age. Similar to the present study, the observed differences in neonatal responses to P4 are not known, but could be due to strain differences in steroid receptor expression or steroid metabolism.

Microarray analysis revealed that P4 treatment suppressed cell cycle and DNA replication pathways in the neonatal mouse uterus. Immunostaining for MKI67 revealed that P4 treatment of neonatal mice decreased the number of proliferating cells in the LE and increased them in the stroma of the uterus, which is similar to a previous study using radioactive thymidine labeling [23]. Furthermore, the inhibitory effect of P4 on uterine epithelial DNA synthesis is mediated by stromal PGR, because epithelial PGR are neither necessary nor sufficient for P4

TABLE 1. Pathways stimulated by progesterone treatment.

Term	*P* value	Molecules
ECM receptor interactions	3.4E-6	*RASSF5, TRAF1, BMP4, FZD7, LAMC3, LAMA1, LAMA2, THBS1, THBS3, VTN*
Calcium signaling pathway	3.4E-3	*HTR2B, ATP2B3, AVPR1A, CACNA1D, CAMK2D, EDNRA, PDGFRA, PTGFR, RYR3, TACR1, TNNC2*
Focal adhesion	4.4E-3	*COL3A1, ITGA4, ITGNB3, ITGNB4, LAMC3, LAMA1, LAMA2, PDGFRA, THBS1, THBS3, VTN*
Cardiomyopathy	5.2E-3	*CACNA1D, CACNA2D1, ITGA4, ITGB3, ITGB4, LAMA2, MYH6*
p53 signaling pathway	1.0E-2	*CCNG2, IGFBP3, RPRM, SERPINE1, SESN1, THBS1*
Complement and coagulation cascades	1.4E-2	*SERPINA1E, PROS1, SERPINA1A, SERPINA1D, SERPINE1, THBD*
Vascular smooth muscle contraction	2.7E-2	*AVPR1A, CACNA1D, EDNRA, KCNU1, KCNMA1, PPP1R14A, RAMP1*
Tryptophan metabolism	4.1E-2	*AOX1, CYP1B1, INMT, MAOB*
Neuroactive ligand receptor interaction	6.1E-2	*HTR2B, AVPR1A, DRD4, EDNRA, GRIA4, PTGER4, PTGFR, ADRA2C, TACR1, VIPR2*
Small cell lung cancer	8.0E-2	*TRAF1, LAMC3, LAMA1, LAMA2, RARB*
TGF-beta signaling pathway	8.5E-2	*BMP4, FST, GDF5, THBS1, THBS3*
Pathways in cancer	9.0E-2	*RASSF5, TRAF1,BMP4, FZD7, LAMC3, LAMA1, LAMA2, PDGFRA, RARB, WNT11, ZBTB16*

TABLE 2. Pathways suppressed by progesterone treatment.

Term	*P* value	Molecules
Pyrimidine metabolism	6.5E-4	*DCTD, DUT, ENTPD8, POLA1, POLR1E, POLR3G, RRM1, NME1, UPB1, UMPS*
Cell cycle	1.4E-3	*E2F2, E2F3, CDC6, CCNB3, CCNE1, MCM2-6, ORC1L, PRKDC*
DNA replication	1.7E-3	*FEN1, MCM2-6, POLA1*
Glycine, serine, and threonine metabolism	8.1E-3	*CHDH, GATM, PHGDH, SHMT1, PSAT1*
Glutathione metabolism	9.6E-3	*GCLC, GSTM6, HNRPII, ODC1, RRM1, GSR*
Arginine and proline metabolism	4.4E-2	*ARG2, GATM, OAT, ODC1, PRODH*
Ether lipid metabolism	5.7E-2	*PPAP2C, PLA2G5, PLA2G7, PLAG12A*
Steroid biosynthesis	6.7E-2	*DHCR24, FDFT1, SQLE*
Cell adhesion molecules	9.4E-2	*CDH4, CLDN6, CNTNAP2, H2-K1, NRXN3, OCLN, PTPRF, SPN*

inhibition of estrogen-induced epithelial DNA synthesis [39]. Collectively, these results indicate that P4 ablation of endometrial adenogenesis in the neonatal mouse uterus may be attributed, in part, to negative effects on proliferation of the LE cells that serve as the progenitors of GE cells. Moreover, the induction of stromal cell proliferation would disrupt normal patterns of cell-cell communication that regulate uterine morphogenesis [3, 15, 25, 40].

In the current study, P4 treatment of neonatal mice markedly upregulated *Ihh*. IHH is a critical mediator of P4 signaling in the uterus during mouse embryo implantation that is expressed in the LE [41]. Overexpression of smoothened (SMO), a downstream effector of IHH expressed in the stroma, disrupted postnatal uterine morphogenesis leading to a hypertrophic uterus with reduced number of uterine glands [42]. Uteri of those mice overexpressing SMO contained increased levels of genes involved in chondrocyte differentiation, such as laminin alpha 2 (*Lama2*) and nidogen 1 (*Nid1*), as well as cartilage-specific genes including the hyaluronan synthase *Has2*. In the present study, P4 treatment of neonatal mice up-regulated those genes in the uterus. Indeed, P4 induction of IHH in the LE would activate SMO pathways in the stroma and presumably inhibit gland development [42].

Microarray analysis revealed that a number of genes involved in ECM-receptor interactions and focal adhesions (e.g., vitronectin, laminin alpha 1, integrin beta 4) were increased by P4 treatment. Endometrial gland morphogenesis involves cell-cell, epithelial-stromal and cell-ECM interactions [3, 5, 14, 15, 43]. In the neonatal mouse uterus, *Wnt4*, *Wnt5a*, and *Wnt16* are expressed in the stroma, while *Wnt7a* and *Wnt11* are expressed in the uterine epithelium [2, 34]. Thus, WNTs likely govern the epithelial-stromal interactions involved in prenatal and postnatal uterine morphogenesis. Recently, conditional deletion of *Wnt4* and *Wnt7a* in the uterus after birth using the PGR-Cre mouse model confirmed their biological roles in postnatal uterine development [20, 44]. Mice deficient in *Wnt4* and *Wnt7a* displayed reduced or completely absent endometrial glands, respectively. In the present study, P4 treatment downregulated *Wnt7a* and *Wnt4* as well as *Wnt16* expression in the uterus. *Wnt16* is expressed in the endometrial stroma of the neonatal and adult mouse uterus [34, 45], but the effect of postnatal deletion of *Wnt16* has not been reported. Of particular note, P4 treatment increased the expression of *Sfrp4*, a secreted inhibitor of Wnt signaling [46], in the present study. In the neonatal ewe lamb, estrogen upregulation of SFRP2 in the uterus was implicated in the effect of that steroid to inhibit endometrial gland development [47]. In the present study, *Msx1* and *Msx2* expression in the uterus were also decreased by neonatal P4 treatment. The ability of the mesenchyme to induce or maintain *Msx1* expression in the Müllerian duct epithelium is thought to involve mesenchymal expression of *Wnt5a* [36], and *Msx2* is required for expression of *Wnt7a* [48]. However, recent results indicate that postnatal deletion of MSX1 or MSX2 or both has no effect of endometrial adenogenesis [49]. Collectively, available results strongly support the idea that WNTs are important epithelial and stromal derived factors that regulate cell proliferation and differentiation in the developing neonatal uterus of the mouse and other species [5].

In addition to the secreted WNTs, other factors play important roles in epithelial proliferation, differentiation and branching morphogenesis in many developing organs including the uterus [3, 50, 51]. *Hgf* and *Met* were found to be expressed by endometrial epithelium and stroma in the adult mouse uterus, and their tissue-specific expression was tightly controlled by steroidal hormones throughout the estrous cycle [52]. Interestingly, P4 stimulated their expression in the stroma, whereas it inhibited their expression in the epithelia [52]. Similarly in the present study, P4 treatment increased *Hgf* expression and decreased expression of *Met* in the neonatal mouse uterus. Because *Fgfr2* or *Met* null mice are embryonically lethal, it is difficult to draw conclusions on FGFs and HGF signaling role in the postnatal uterus. However, injections of FGF7 into neonatal mice stimulated proliferation of the uterine LE [16]. Recently, Li et al. [17] found that P4 inhibits expression of several FGFs (*Fgf1*, *Fgf2*, *Fgf9*, *Fgf18*) in the stroma of the adult uterus, thereby reducing LE cell proliferation. In the present study, microarray analysis found that P4 treatment of neonatal mice reduced *Fgf9* and *Fgf12* expression in the uterus along with LE cell proliferation. Thus, FGF and HGF signaling are also likely important for uterine development and adenogenesis as in other organs exhibiting branching morphogenesis such as the lung, mammary gland and kidney [53–55].

Matrix metalloproteinases (MMPs) and their inhibitors are involved in postnatal uterine morphogenesis in the mouse [56–58] by reorganizing the extracellular matrix necessary for endometrial gland development [37]. Hu et al. [57] implicated the MMP system (*Mmp2*, *Mmp7*, *Adamts1*) in the postnatal uterine morphogenesis in the mouse. In the present study, P4 disruption of endometrial adenogenesis was associated with a reduction *Mmp7* and *Mmp2* expression. Further, *Car2* was reduced in P4-treated mice on PD 5 and 10. Carbonic anhydrases (CAR) are hypothesized to have a key role in regulating neonatal mouse uterine adenogenesis by affecting GE cell migration and MMP activity [38]. Interestingly, inhibition of CAR2 activity in neonatal mice reduced endometrial gland development [38]. Collectively, available results support the idea that P4 deregulates MMP expression and activity in the endometrium, which impairs ECM remodeling required for GE cell proliferation, migration and/or differentiation in the developing neonatal uterus.

A recent study found that conditional ablation of *Foxa2* in the postnatal mouse uterus using the PGR-Cre model resulted

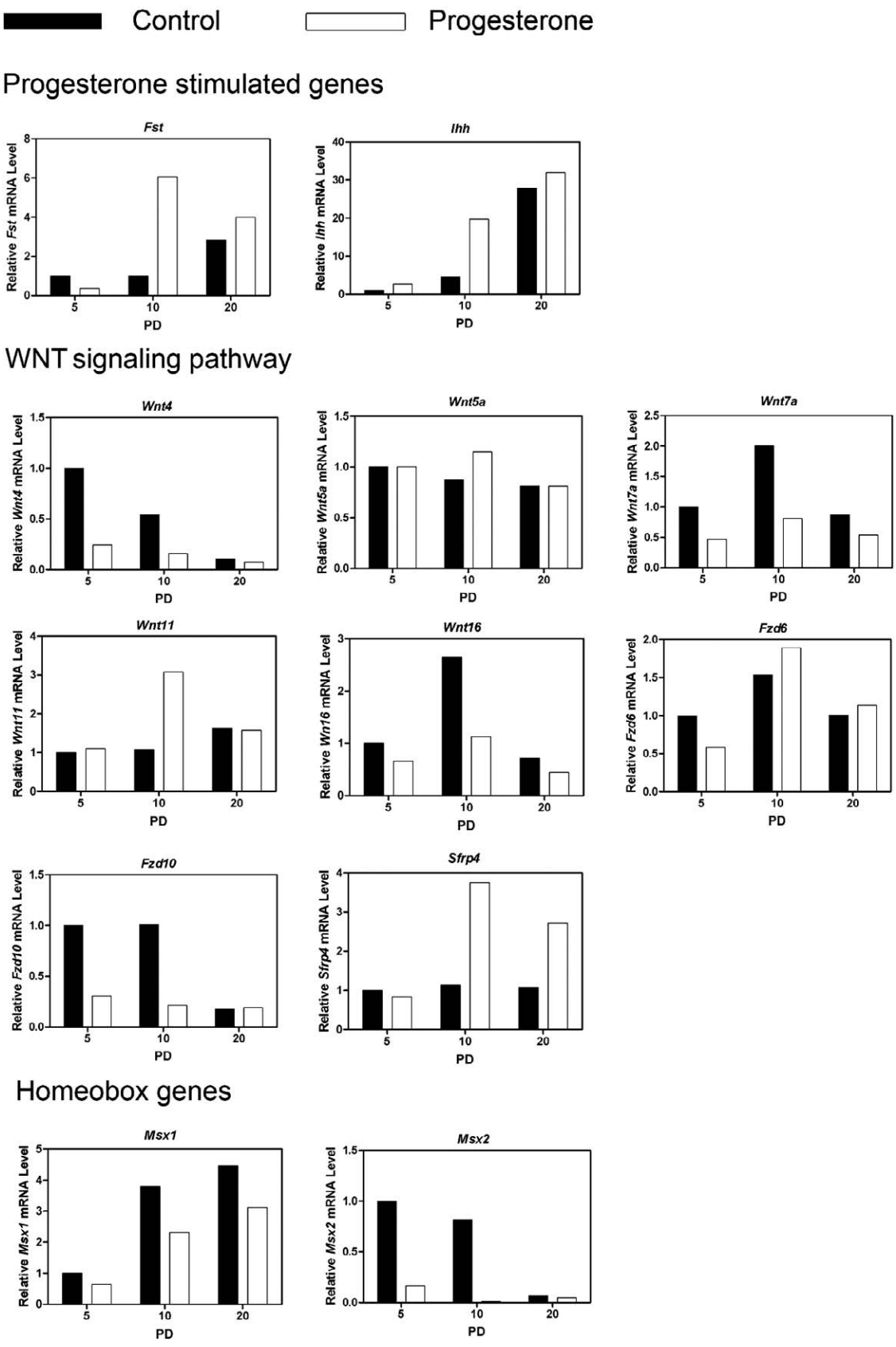

FIG. 4. Progesterone alters expression of progesterone stimulated, WNT signaling and homeobox genes in the neonatal mouse uterus. Mice were treated with sesame oil vehicle as a control or P4 in vehicle from PD 2 to 10 after birth. The relative mRNA levels of the indicated genes was measured in the uterus of control and P4-treated mice by real-time RT-PCR. Data are presented as fold change relative to the mRNA level on PD 5 in uteri from control mice.

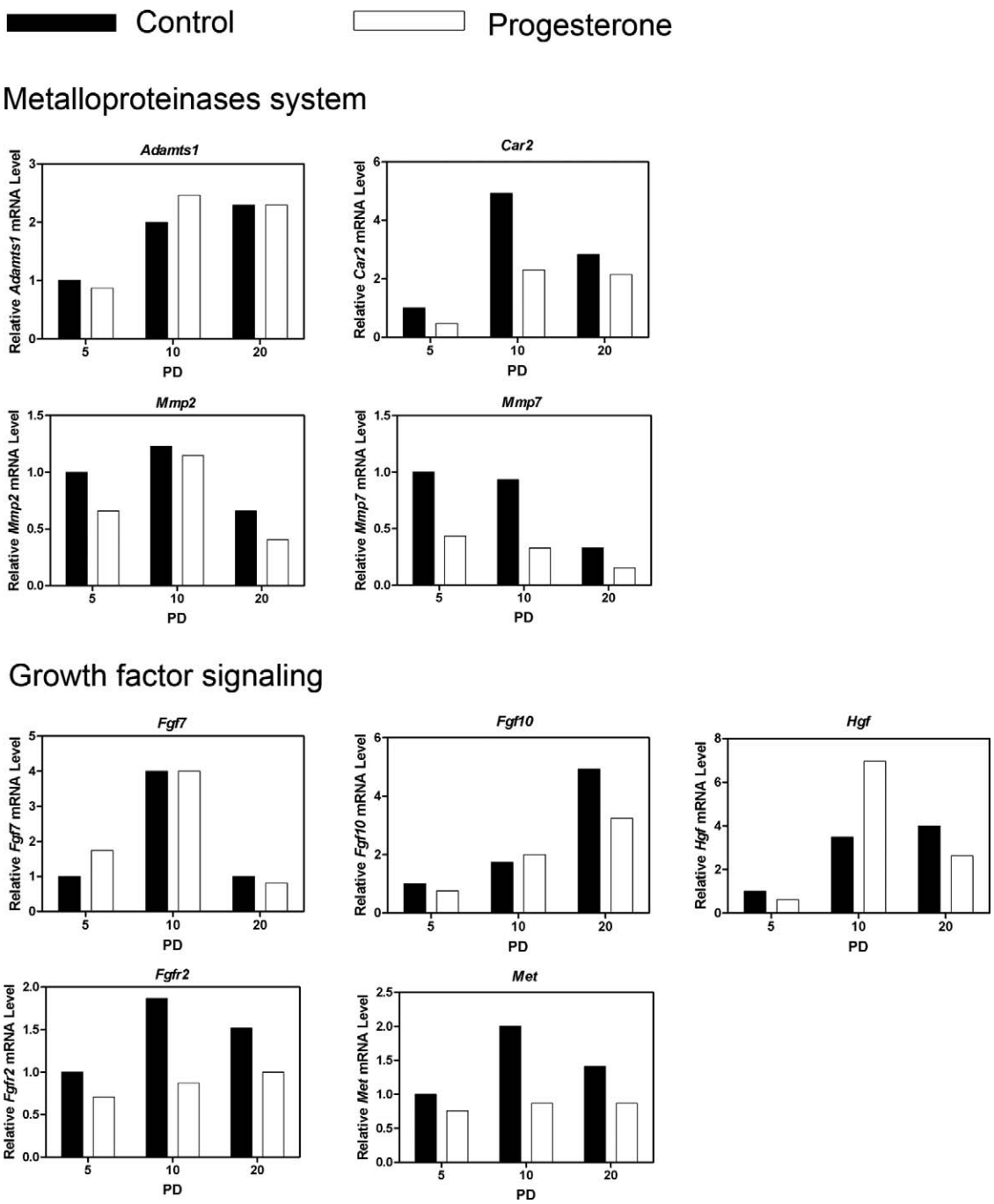

FIG. 5. Progesterone alters expression of the metallproteinase system and growth factor signaling genes in the neonatal mouse uterus. Mice were treated with sesame oil vehicle as a control or P4 in vehicle from PD 2 to 10 after birth. The relative mRNA levels of the indicated genes was measured in the uterus of control and P4-treated mice by real-time RT-PCR. Data are presented as fold change relative to the mRNA level on PD 5 in uteri from control mice.

in an absence of endometrial glands in the adult, suggesting that FOXA2 is a critical regulator of endometrial adenogenesis in the mouse uterus [19]. In the lung and prostate, *Foxa2* also regulates epithelial budding and morphogenesis [59, 60]. The present study found that *Foxa2* is a sensitive marker of GE differentiation and development, because *Foxa2* expression increased with age in control but not P4-treated mice that lack endometrial glands. The nuclear localization of FOXA2 in the endometrial GE cells is similar to other reports that *Foxa2* is predominantly expressed by GE cells [19, 20] and consistent with its function as transcription factor regulating organogenesis [61].

In summary, the present study illustrates the utility of P4 as a tool to discover mechanisms regulating postnatal uterine development and provides insights into P4 action within the uterus. Available results support the hypothesis that P4 inhibits endometrial adenogenesis in the developing neonatal uterus by altering expression of morphoregulatory genes and consequently disrupting normal patterns of cell proliferation and communication. Future mechanistic studies are needed to more

precisely understand the novel factors and pathways involved in endometrial adenogenesis in the neonatal mouse uterus, which is important as endometrial glands are required for fertility of the adult.

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