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Effect of Pregnancy and Delivery on Cytokine Expression in a Mouse Model of Pelvic Organ Prolapse

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Abstract

Objectives—The aim of this study was to determine the effect of pregnancy and delivery mode on cytokine expression in the pelvic organs and serum of lysyl oxidase like-1 knockout (*Lox1* KO) mice, which develop pelvic organ prolapse (POP) after delivery.

Methods—Bladder, urethra, vagina, rectum and blood were harvested from female *Lox1* KO mice during pregnancy, after vaginal or cesarean delivery, and from sham cesarean and unmanipulated controls. Pelvic organs and blood were also harvested from pregnant and vaginally delivered wild-type (WT) mice and from unmanipulated female virgin WT controls. Specimens were assessed using qRT-PCR and/or ELISA.

Results—Both *Cxcl12* and *Ccl17* mRNA were significantly upregulated in the vagina, urethra, bladder, and rectum of pregnant *Lox1* KO mice compared to pregnant WT mice, suggesting systemic dysregulation of both of these cytokines in *Lox1* KO mice as a response to pregnancy. The differences in cytokine expression between *Lox1* KO and WT mice in pregnancy persisted

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Conflicts of Interest: Dr. Penn has an interest in Juventas, Inc., which has licensed his SDF-1 related technology. Dr. Damaser has a patent pending on stem cell therapeutics for genitourinary dysfunction. The other authors have no real or potential conflict of interest with the results of this study.

after vaginal delivery. *Ccl7* gene expression increases faster and to a greater extent in *Lox11* KO mice, translating to longer lasting increases in CCL7 in serum of *Lox11* KO mice after vaginal delivery, compared to pregnant mice.

Conclusions—*Lox11* KO mice have an increased cytokine response to pregnancy, perhaps because they are less able to reform and re-crosslink stretched elastin to accommodate pups and this resultant tissue stretches during pregnancy. Upregulation of CCL7 after delivery could provide an indicator of level of childbirth injury, to which the urethra and vagina appear to be particularly vulnerable.

Keywords

Pelvic organ prolapse; vaginal delivery; mice; LOXL-1; inflammatory cytokines

Introduction

Pelvic organ prolapse (POP) is the descent of one or more pelvic organs into the vagina. Although not life-threatening, POP significantly reduces quality of life due to bulge symptoms, sexual dysfunction, and social embarrassment.¹ The incidence of POP increases with age, peaking at 71–73 years old with an incidence of 4.3 per 1000 women, leading to a 12% lifetime risk of undergoing surgery.²

Aberrant connective tissue metabolism, genetic factors, obesity, advanced age, pregnancy and vaginal delivery are among the most important risk factors for POP.¹ Maternal injuries of childbirth are believed to contribute significantly since increased length of 2nd stage of labor, large newborn weight, and multiparity increase the risk of POP.³ Previous studies have used imaging assessments of patients and mechanical models to describe the extent of anatomical injuries to the maternal pelvic floor sustained during childbirth in women.^{4–6} However, little research has been conducted to elucidate molecular markers for maternal injury in genitourinary tissues during the peripartum period that might provide insight into the mechanisms of injury that could lead to POP.^{7–9}

Animal models have become invaluable for investigating the role of pregnancy and parturition in development of POP.¹⁰ Although, no animal model is capable of incorporating all the characteristics and risk factors of POP in women, lysyl oxidase like-1 knockout (*Lox11* KO) mice develop POP with phenotypic characteristics and risk factors similar to women, although they present a higher incidence of rectal prolapse than humans.^{11–14} LOXL1 is a metalloenzyme responsible for crosslinking tropoelastin into its functional form, elastin, which provides the extracellular matrix (ECM) with its structure and recoil properties.¹⁵ Similar to women, parity is the leading risk factor for POP in *Lox11* KO mice and POP is delayed, but not prevented, by cesarean delivery.^{11,12} In addition, the prolapse rate in *Lox11* KO mice increases with age, and presents with variable prolapse grades and incidences (50–80%).^{16,17}

Previous work has demonstrated that stem cell homing cytokines become upregulated after injury in a variety of different tissues.^{18–23} The exact pathway for the regulation of these cytokines is still unclear. Nevertheless, previous studies have shown that targeting the

modulation of cytokines and their receptors at different time points after acute injury could be useful for development of novel therapeutics.^{18,23,24} Chemokine C-C motif ligand 7 (CCL-7), formerly known as monocyte-specific chemokine 3 (MCP-3), and chemokine C-X-C motif ligand 12 (CXCL-12), formerly known as stromal cell-derived factor 1 (SDF-1) function as signaling molecules to recruit circulating progenitors and stem cells, facilitating recovery from injury.^{20,21,24} CCL-7 is responsible for recruiting hematopoietic and mesenchymal stem cells to the site of injury, where the mesenchymal stem cells induce fibroblasts to remodel ECM.²⁰ CXCL-12 signals for hematopoietic and endothelial stem cells, contributing to angiogenesis in the area of injury.²¹

We have previously investigated upregulation in CCL-7 and CXCL-12 in rat pelvic organs after ischemia and in other injury models.^{7,9,20,21,25} Given the response of these cytokines to injury, they could be used as potential injury markers as they are mediators of inflammatory response.^{8,18,23} We hypothesize that pregnancy and delivery mode will differentially affect the concentration and expression of CCL-7 and CXCL-12 both in the pelvic organs and systemically in *LoxII* KO and wildtype (WT) mice. The primary aim of the present study was to determine the differential effect of pregnancy and delivery mode on CCL-7 and CXCL-12 expression in the pelvic organs and serum of *LoxII* KO and WT mice. The secondary aims were to determine changes in expression with pregnancy and pup delivery in *LoxII* KO and WT mice and assess differential expression between cesarean and vaginal delivery in *LOXLI* KO mice.

Materials and Methods

Animal Breeding Techniques and Tissue Harvest

All work was performed with the approval of the Cleveland Clinic Institutional Animal Care and Use Committee (IACUC). One hundred twenty-six age-matched *LoxII* KO female mice were bred at 8–9 weeks of age. The presence of a vaginal plug was used to approximate gestational day 1. Mice were then randomized into 3 groups: vaginal delivery, cesarean delivery, and pregnant. Bladder, urethra, vagina, rectum, and serum were harvested at the following time points: 15 minutes, 1, 4, 12, 24, 48 hours, or 4, 7 days after injury (vaginal delivery or cesarean-Figure 1A). These time points were selected based on the timecourse of cytokine expression in previous studies.^{7,9,22,25,26} *LoxII* KO mouse gestation duration is approximately 21 days, so, as a feasible time point for studying the effects of pregnancy in the absence of delivery as a control for delivered mice, tissues were harvested on the 20th day of gestation, prior to parturition for the pregnant group (Figure 1A). Sixty-four additional age-matched virgin *LoxII* KO female mice were utilized as sham cesarean injury controls or unmanipulated virgin controls (Figure 1A). These animals were not placed with male breeders but were housed under identical conditions with a breeder diet, and had organs harvested at the same time points. All procedures were performed with animals under isoflurane anesthesia.

Twenty-eight wild type (WT) female mice (Jackson Laboratory hybrid C57B1/6 and Sv129) were bred at 8 weeks old. Pelvic organs and serum were harvested on the 20th day of gestation or 1, 24, or 48 hours after vaginal delivery (Figure 1B), selected based on the data from *LoxII* KO mice as the seminal time points for comparison. One additional animal at

each timepoint had serum harvested only due to problems with organ harvest. Pelvic organs and serum was also harvested from 7 additional age-matched virgin WT unmanipulated control females for comparison (Figure 1B).

Cesarean delivery of *LoxH* KO mice was performed as modified from our previous work.¹¹ WT mice did not undergo Cesarean delivery. For Cesarean delivery, the abdomen was entered in layers under 2% isoflurane anesthesia, through a transverse incision between the 1st and 2nd pair of nipples. The uterus was exteriorized and multiple incisions were made on the anti-mesenteric side of each uterine horn. One to three pups, each with its placenta, were removed through each incision. Uterine incisions were closed with single stitches, and the abdominal incision was closed in a running fashion (6-0 silk). The vagina was not manipulated during cesarean delivery. For sham cesarean injury, both non-pregnant uterine horns were exposed and a single incision was made on each horn. Uterine and abdominal incisions were closed as described above. Postoperative subcutaneous buprenorphine (0.1mg/kg) was given to animals in cesarean and sham-cesarean groups twice a day for 48 hours.

Pelvic organs were harvested under a surgical microscope via a midline abdominal incision after which the pubic symphysis was opened for complete urethral visualization. The bladder and urethra were dissected from the anterior vaginal wall, and then the urethra was transected from the bladder. The vagina was dissected from the rectum and transected proximally at the cervix level and distally at the perineal skin. The rectum directly underlying the vagina was also harvested. Blood samples were obtained by direct cardiac aspiration, and were allowed to clot prior to being centrifuged at 5000 rpm for 15 minutes. The supernatant (serum) was aspirated and stored at -80°C. All harvested tissues were stored at -80°C until tissue digestion for RNA isolation for quantitative real time reverse transcription polymerase chain reaction (qRT-PCR) or enzyme-linked immunosorbent assay (ELISA).

Gene Expression Analysis

Frozen tissues (n=6–7 per time point in each group; Figure 1) were ground in liquid nitrogen, and total RNA was isolated using the RNeasy®-4PCR kit (Life Technologies, Grand Island, NY #AM1914) according to the manufacturer's protocol. RNA concentration was determined using a NanoDrop® ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE). Reverse transcription reactions were performed in 20 µl of reaction volume with 300 ng of total RNA, random primers, and 1 unit of reverse transcriptase at 25°C for 10 minutes, 37°C for 120 minutes and 85°C for 5 minutes (High-Capacity cDNA Reverse Transcription Kit, #4368814, Life Technologies). Twenty-five ng of input cDNA was used in each 25 µl reaction for qRT-PCR (TaqMan Gene Expression Master Mix: PN 4369016, ABI Prism 7500, Life Technologies). Mouse CCL-7 (#Mm00443113_m1) and CXCL-12 (#Mm00445552_m1) primer-probes were obtained from Life Technologies. The endogenous control was 18S (#4319413), and the standard curve method was used to determine the relative amount of the target genes.

Tissue Protein Analysis

Due to the amount of tissue needed to perform biochemical analysis, RNA isolation for qRT-PCR and ELISA were assessed in different animals (Figure 1A). Frozen tissues were crushed in liquid nitrogen. Whole tissue extracts were prepared using a solution composed of 20 mM Tris-HCl, pH7.5, 150mM sodium chloride (NaCl), 1 mM disodium ethylenediaminetetraacetic acid (Na₂EDTA), 1 mM ethylene glycol tetraacetic acid (EGTA), 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na₃VO₄, protease inhibitor cocktail (Roche, Basel, Switzerland #11697498001) and 1 mM phenylmethylsulfonyl fluoride (PMSF) added to the frozen crushed tissues which were then homogenized. Tissue lysates were kept on ice at 4°C for 20 minutes and then centrifuged at 13000 rpm for 15 minutes at 4°C. The concentration of total protein was estimated by Bradford protein assay (Bio-Rad, Hercules, CA #500-0006) using bovine serum albumin (BSA, Life Technologies # 15561020) as a standard. One-hundred μ g per sample of whole tissue extracts were tested using ELISA kits for CCL-7 and CXCL-12 (#BMS6006INST, eBioscience, San Diego, CA) according to the manufacturer's protocol.

Serum Protein Analysis

Serum samples were thawed slowly at 4°C. Concentration of CCL-7 in serum was quantified using 25 μ l of serum with duplicate wells for each sample, using the same ELISA kits as above.

Data Analysis

Gene expression data is presented as mean $\pm$ standard error of the mean (SEM) for relative expression normalized to the endogenous control. SEM was utilized as it is appropriate when comparing populations, as in this study. Sample size analysis indicated that 6–8 animals/group would be sufficient to test the hypothesis at multiple time points. The data was analyzed using mixed model regression. After the mixed models analysis, posthoc comparisons were made using Dunnett's, Bonferroni, or Students' t-test to identify significant differences between individual groups as appropriate since multiple post-hoc tests for mean difference significance testing were needed to address different aspects of the analysis. Dunnett's post hoc test was used to compare delivery type outcomes (after vaginal delivery, cesarean delivery, or sham cesarean injury) within *Lox11* KO and WT strains to pregnant animals. The Bonferroni post hoc test was used to assess specific contrasts between delivery types across time. The Students' t-test was used to compare *Lox11* KO versus WT outcomes at the same time point. $P < 0.05$ was used in all cases to indicate a statistically significant difference between groups. Since there are many comparisons in this study, for clarity we only report if the difference was statistically significant with $P < 0.05$ or not.

Results

Differential expression of *Cxcl12* and *Ccl7* between WT and KO mice

Compared to virgin WT mice, *Cxcl12* mRNA was significantly upregulated in the vagina of virgin *Lox11* KO mice but not in the urethra, bladder, or rectum (Figure 2). *Ccl7* mRNA, on the other hand, was significantly upregulated in the rectum of virgin *Lox11* KO mice, but not

in the vagina, urethra, or bladder (Figure 3). Both *Cxcl12* and *Ccl7* mRNA were significantly upregulated in the vagina, urethra, bladder, and rectum of pregnant *Lox11* KO mice compared to pregnant WT mice.

The differences in cytokine expression between *Lox11* KO and WT mice in pregnancy persisted after vaginal delivery at the first two time points investigated in both *Lox11* KO and WT mice (1 and 24 hours). *Cxcl12* mRNA was significantly increased in the vagina, urethra, bladder, and rectum 1 and 24 hours after delivery in *Lox11* KO mice compared to WT mice (Figure 2). Although 48 hours after delivery, *Cxcl12* expression was decreased in the urethra of *Lox11* KO mice compared to WT mice, *Cxcl12* mRNA was significantly upregulated in the bladder and rectum. As with *Cxcl12*, *Ccl7* mRNA was significantly upregulated in the vagina, urethra, and rectum 1 and 24 hours after delivery in *Lox11* KO mice compared to WT mice, as well as in the bladder of *Lox11* KO mice 24 hours after delivery (Figure 3). Forty-eight hours after delivery, *Ccl7* mRNA was downregulated in the vagina and urethra of *Lox11* KO mice compared to WT mice. Overall, these data illustrate increased cytokine response in *Lox11* KO mice compared to WT mice after vaginal delivery, particularly 1 and 24 hours postpartum.

Vaginal Delivery versus Cesarean Delivery in *Lox11* KO mice

There were no significant differences in *Ccl7* and *Cxcl12* mRNA expression in any of the pelvic organs of *Lox11* KO mice following cesarean compared to sham cesarean, suggesting that any significant differences observed between cesarean and vaginal delivery are due to the route of delivery (Figures 2 & 3). There were no significant differences in *Cxcl12* mRNA expression between cesarean and vaginal delivery in the urethra or vagina. However, *Cxcl12* mRNA in the bladder was increased 15 minutes following cesarean compared to vaginal delivery, but decreased 12 hours following cesarean compared to vaginal delivery (Figure 2).

Cxcl12 mRNA was increased in the rectum 1 hour following vaginal delivery compared to cesarean in *Lox11* KO mice (Figure 2). In contrast to *Cxcl12* for which there were no significant differences between cesarean and vaginal delivery in the vagina, vaginal delivery resulted in significant upregulation of *Ccl7* mRNA 15 minutes and 1 hour postpartum in the vagina and 1 hour postpartum in the urethra compared to cesarean, suggesting increased injury to the vagina as a result of vaginal delivery (Figure 3). However, by 24 hours postpartum, *Ccl7* mRNA was significantly decreased in the vagina after vaginal delivery compared to cesarean.

Similar to *Cxcl12* mRNA expression in the bladder, *Ccl7* mRNA expression was increased after cesarean compared to vaginal delivery in the bladder of *Lox11* KO mice 1 and 4 hours after delivery (Figure 3). Moreover, *Ccl7* mRNA was increased in the rectum 12 hours after vaginal delivery compared to cesarean.

Analysis of serum revealed no baseline differences between WT and *Lox11* KO mice in virgins or pregnant mice (Figure 4). There was a significant increase in CCL7 concentration 1, 4, 12, 24, and 48 hours after cesarean compared to vaginal delivery (Figure 4), likely a result of cytokines being secreted into the blood supply from tissues injured during cesarean

surgery (e.g. skin, rectus abdominis, peritoneum, etc). However, there were also significant differences between cesarean and sham cesarean at all these time points.

Effect of Pregnancy on *Cxcl12* and *CCL7* in *Lox11* KO and WT mice

Cxcl12 mRNA expression is significantly decreased in the vagina, bladder, and rectum of pregnant WT mice but only in the vagina of pregnant *Lox11* KO mice compared to virgin mice (Figure 2). In contrast to *Cxcl12*, *Ccl7* mRNA is upregulated in the urethra only of both pregnant *Lox11* KO and WT mice compared to virgin mice, although to a significantly greater extent in *Lox11* KO mice (Figure 3). CCL7 protein concentration was significantly increased in the vagina, urethra, bladder and rectum of pregnant *Lox11* KO mice compared to control (Figure 4). However, these differences do not translate to differences in serum protein of CCL7 in either *Lox11* KO or WT mice.

Effect of Delivery on *Cxcl12* and *CCL7* in *Lox11* KO and WT mice

Compared with pregnant *Lox11* KO mice, *Cxcl12* mRNA is significantly increased 1 hour after vaginal delivery in the urethra and 24 hours after vaginal delivery in the vagina (Figure 2). In contrast, *Cxcl12* mRNA is not significantly increased in the urethra and is significantly increased in the vagina of WT mice 48 hours after vaginal delivery compared to pregnant mice (Figure 2). This supports the results noted above that the increase in *Cxcl12* gene expression in the urethra and vagina after vaginal delivery is faster and greater in *Lox11* KO mice than in WT mice. Although *Ccl7* gene expression significantly increases in the vagina, urethra, bladder and rectum of WT mice as soon as 1 hour after vaginal delivery compared to WT pregnant mice, it increases faster and to a greater extent in the vagina and bladder of *Lox11* KO mice (15 minutes), similar to the pattern observed with *Cxcl12* expression (Figure 3). CCL7 had a longer lasting increase in the serum of *Lox11* KO mice after vaginal delivery than in WT mice, compared to pregnant mice (Figure 4).

Discussion

Current treatments for POP do not target the pathophysiology of the disease and surgery is the most frequent management option, often leading to recurrence and sometimes requiring surgical revision.²⁷ Animal models, although imperfect, are crucial to understanding the pathophysiology of POP, assessing markers indicative of childbirth-induced pelvic floor injury, and testing novel therapeutics.¹⁰ Research with the *Lox11* KO mouse model in particular is supported by human genetic data implicating abnormal elastin homeostasis in the pathophysiology of POP.^{14,28} In addition, POP in *Lox11* KO mice simulates the phenotype of POP in women, with vaginal delivery as a major risk factor, often affecting voiding function, and progressing with age.^{1,11,12}

We and others have previously shown that expression and concentration of inflammatory cytokines, such as CCL7 and CXCL12, are altered in response to different levels of tissue injury in animal models and humans.^{8,18,20–23,25} Although we did not specifically investigate which cell types in pelvic organs produced these cytokines, it is known that smooth muscle cells and fibroblasts secrete these cytokines in response to injury and other manipulations.^{29–31} Since these cell types abound in pelvic organs, we presume that it is

smooth muscle cells and fibroblasts that secreted CCL7 and CXCL12 in our study but confirmation of that is beyond the scope of this study.

In this study, we found that baseline levels (i.e. in virgin mice) of *Cxcl12* mRNA in the vagina were significantly higher in *Lox11* KO mice compared to WT mice. Although not statistically significant, *Cxcl12* was also increased in the other pelvic organs in virgin KO mice. Therefore, differences in *Cxcl12* expression between KO and WT mice at further time points may be due to the higher levels of *Cxcl12* at baseline and not necessarily due to alterations in tissue response to pregnancy and parturition.

In contrast, *Ccl7* mRNA expression in virgin KO and WT mice was remarkably similar in the vagina and pelvic organs (except for the rectum). Given that the pathogenesis of POP in *Lox11* KO mice is dependent on parity, differences in antepartum and postpartum *Ccl7* expression between KO and WT mice could indicate altered response to or degree of tissue injury. Interestingly, pregnancy resulted in a significant increase of both *Ccl7* and *Cxcl12* in KO mice compared to WT mice, a finding that was consistent among all the pelvic organs analyzed. During pregnancy, pelvic tissues undergo significant remodeling in preparation for childbirth, a process in which LOXL1 and its absence has a significant impact.^{16,32–35} For example, matrix metalloproteinase-2 (MMP2), which has been shown to modulate CCL-7, is upregulated late in pregnancy by high levels of estrogen and progesterone.³⁶ Therefore the increase in *Ccl7* and *Cxcl12* expression in KO mice could be due to differences in tissue remodeling between the two genotypes due to *Lox11* deficiency.

Not surprisingly, *Ccl7* expression in the vagina was significantly upregulated early postpartum (0.25 and 1 hr) in vaginal delivery compared to Cesarean delivery in KO mice, suggesting that vaginal delivery induces an injury tissue response even in mice, which, unlike women, generally do not have difficult labors. Although we did not study Cesarean delivery in WT mice in this study, there was also a significant increase in *Ccl7* expression in vaginas of WT mice after delivery compared to pregnant mice, although this difference was not as great as in *Lox11* KO mice, suggesting a lesser injury repair response in WT mice. These data also help further explain our previous finding in *Lox11* KO mice showing that vaginal delivery results in higher rates of POP development compared to Cesarean delivery.¹¹

Ccl7 was also upregulated in the urethra after vaginal delivery compared to Cesarean delivery in KO mice. This finding supports our previous work using a simulated childbirth injury model for pelvic floor dysfunction in rats.^{25,26} *Ccl7* mRNA expression is upregulated in the urethra 1 hour after vaginal distension in rats, suggesting that this finding could also be related to ischemia and tissue trauma, not pregnancy or labor alone.^{22,25} Additionally, this data helps further explain our previous study of lower urinary tract function in *Lox11* KO mice in which we showed that parity significantly contributed to lower leak point pressures.¹² Overall, our data suggest that *Lox11* deficiency results in aberrant antepartum and postpartum gene expression responses to pregnancy and parturition that may be exacerbated by vaginal delivery.

In contrast to the vagina and urethra, the bladder presented consistently elevated levels of *Ccl17* in animals that underwent surgical procedures (cesarean and sham cesarean). This is most likely a result of urinary retention leading to bladder wall distention and a gene expression response to the resultant injury from anesthesia which suppresses voiding reflexes or use of an opioid-derived postoperative analgesic.^{37,38} Mice were not catheterized during surgery and, although beyond the scope of this study, this would represent a method to test this mechanism for upregulated *Ccl17* in the bladder after cesarean and sham cesarean.

Serum levels of CCL7 in KO and WT mice were not significantly different between pregnant and virgin groups. However, CCL7 serum levels in both KO and WT mice were significantly upregulated after vaginal delivery and after Cesarean delivery in KO mice, compared to the pregnant groups, which shows that parturition-induced tissue injury is detectable in serum. We also found a significant increase in serum levels of CCL7 after Cesarean delivery compared to vaginal delivery in *Lox11* KO mice. Given that Cesarean delivery results in significant tissue injury as a result of surgery, this finding supports the use of CCL7 as a marker for tissue injury.

Vaginal delivery is a major risk factor for POP, but pregnancy also plays an important role.¹ Hormonal changes during pregnancy affect physiological parameters such as maternal circulation as well as tissue biomechanics and composition of the uterus and vagina,³⁹ some of which are mediated via MMPs, which contribute to ECM remodeling.^{36,40} One limitation of this work is that we focused on multiple time points after delivery and only one reproducible time point during pregnancy. Therefore, we may have missed variations in cytokine expression during gestation. In addition, we focused on organs most affected by POP and did not investigate cytokine expression in the uterus and cervix, since we expected cytokine expression in these organs to be dominated by surgical manipulation of cesarean delivery. We also did not investigate other organs not affected by POP.

Due to the limited amount of tissue available from each mouse, particularly from the very small mouse urethra, we only assessed two cytokines in this study, although many others could have been considered for investigation. Future studies could be designed to investigate other cytokines. In addition, rather than using multiple endogenous control genes as we might have, we used only a single housekeeping gene (18S) for RT-PCR studies, which we have found to provide greater reliability with this model than other endogenous control genes.

Likewise, due to the small amounts of tissue available, we did not perform histological or functional lower urinary tract analyses and therefore do not know whether upregulation of cytokines correlates with function or structural changes in tissues. We aimed to characterize the population of *Lox11* KO mice and compare them to the population of WT mice in the expectation that these population-based outcomes could be utilized for comparison to structural and functional assays in future studies. Nonetheless, previous studies have suggested that pelvic floor injuries caused by delivery in *Lox11* KO mice alter the structure of collagen, elastin, and muscles in the urethra and vagina,^{12,13,17} and as mentioned previously, reduced leak point pressure is correlated with parity in *Lox11* KO mice, which is demonstrative of urethral dysfunction after delivery.^{12, 13}

In conclusion, we have demonstrated that *Cxcl12* and *Ccl17* mRNA expression is altered in *Lox11* KO compared to WT mice. However, alterations in *Cxcl12* expression were not dependent on pregnancy and parturition (as evidenced by differences in virgin animals) and *Ccl17* appears to play a more significant role. Differences between KO and WT groups were primarily contained within the pregnancy and later postpartum time points (i.e. 24 and 48 hours). *Ccl17* mRNA expression was highly affected by mode of delivery as noted by attenuated expression levels in mice undergoing Cesarean delivery. The urethra and vagina appear to be particularly vulnerable to increased cytokine expression after delivery, providing motivation to explore the cascade of molecular and cellular processes in these organs particularly as they relate to women at risk for pelvic floor disorders. It is possible that upregulation of CCL-7 protein concentration in serum after delivery may serve as an indicator of extent of injury, which could help identify women at high risk of pelvic floor disorders later in life. However, further research is needed to test this hypothesis. These data may also aid in development of novel stem-cell-based therapies, where injury-related homing cytokines can be exploited to direct cells to the sites of injury.^{41,42}

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Single Sentence Summary

Lysyl oxidase like-1 knockout mice, which develop pelvic organ prolapse after delivery have an increased cytokine upregulation response to pregnancy, perhaps because they are less able to reform and re-crosslink stretched elastin to accommodate pups, which may be translatable to humans as an indicator of level of childbirth injury.

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Figure 1.

Experimental design flow chart showing *Lox11* KO mice with tissues harvested for mRNA (qRT-PCR) analysis, *Lox11* KO mice with tissues harvested for protein (ELISA) analysis, and *Lox11* KO with blood harvested for protein (ELISA) analysis (**A**); WT mice with tissues harvested for mRNA (qRT-PCR) analysis (**B**) The total number of animals in these charts appears to be higher than the total number of animals in the study since blood was harvested from the same animals that had tissue harvested, but not all blood samples were viable to have serum collected.

Figure 2.

Relative *Cxcl12* gene expression normalized to expression of *18S* in the vagina (A), urethra (B), bladder (C), and rectum (D) of *Lox11* KO and WT mice at different time points after vaginal delivery, cesarean delivery, sham cesarean injury, and in pregnant and unmanipulated virgin mice. **α** indicates a significant difference between unmanipulated virgin *Lox11* KO and WT mice; **β** indicates a significant difference between pregnant *Lox11* KO and WT mice; **#** indicates a significant difference between pregnant and virgin mice of the same genotype; ***** indicates a significant difference between *Lox11* KO and WT vaginal delivered mice at the same postpartum time point; **δ** indicates a significant difference between vaginal or cesarean delivered and pregnant *Lox11* KO mice; **ε** indicates a significant difference between vaginal delivered and pregnant WT mice; **γ** indicates a significant difference between vaginal delivered and cesarean delivered *Lox11* KO mice at the same postpartum time point. In all cases, $p < 0.05$ was used to indicate a statistically significant difference. Each symbol represents the mean $\pm$ standard error of the mean of data from 6–7 mice. Note that the y-axis scales are different for each panel since comparisons between organs were not made.

Figure 3.

Relative *Ccl7* gene expression normalized to expression of *18S* in the vagina (A), urethra (B), bladder (C), and rectum (D) of *Lox11* KO and WT mice at different time points after vaginal delivery, cesarean delivery, sham cesarean injury, and in pregnant and unmanipulated virgin mice. β indicates a significant difference between pregnant *Lox11* KO and WT mice; # indicates a significant difference between pregnant and virgin mice of the same genotype; * indicates a significant difference between *Lox11* KO and WT vaginal delivered mice at the same postpartum time point; δ indicates a significant difference between vaginal or cesarean delivered and pregnant *Lox11* KO mice; ϵ indicates a significant difference between vaginal delivered and pregnant WT mice; γ indicates a significant difference between vaginal delivered and cesarean delivered *Lox11* KO mice at the same postpartum time point. In all cases, $p < 0.05$ was used to indicate a statistically significant difference. Each point represents the mean $\pm$ standard error of the mean of data from 6–7 mice. Note that the y-axis scales are different for each panel since comparisons between organs were not made.

Figure 4.

CCL7 protein concentration in pelvic organs at different time points after vaginal delivery and in pregnant and unmanipulated virgin *Lox11* KO mice (**A**) and in serum of *Lox11* KO and WT mice at different time points after vaginal delivery, cesarean delivery, sham cesarean injury, and in pregnant and unmanipulated virgin mice (**B**). # indicates a significant difference between pregnant and virgin mice of the same genotype; δ indicates a significant difference between vaginal or cesarean delivered and pregnant *Lox11* KO mice; ϵ indicates a significant difference between vaginal delivered and pregnant WT mice; γ indicates a significant difference between vaginal delivered and cesarean delivered *Lox11* KO mice at the same postpartum time point; + indicates a significant difference between cesarean delivered and sham cesarean injury in *Lox11* KO mice at the same time point. In all cases, $p < 0.05$ was used to indicate a statistically significant difference. Each symbol represents the

mean $\pm$ standard error of the mean of data from 6–8 mice. Note that the x- and y-axes scales in **A** and **B** are different.