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Exploring the Roles of Progesterone and Estrogen Receptors in Human Labor

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By:

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Abstract

Integration of RNA-seq and ESR1/PGR ChIP-seq in pregnant human myometrium reveals potential regulators of labor

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Preterm birth (PTB) is the leading cause of infant morbidity worldwide. Approximately 380,000 babies are born prematurely in the USA every year. Estrogen (E2) and progesterone (P4) play essential roles during pregnancy and labor; a clear understanding of their action mechanisms, however, is lacking. E2 and P4 function by activating their cognate nuclear receptors ESR1 and PGR, respectively, to affect their binding to regulatory regions of target genes and control their transcription and function. In this study, I sought to identify steroid hormone target genes and pathways critical for myometrium quiescence and contraction. Elucidating molecular mechanisms whereby the myometrium transforms from a quiescent to a contractile state would fill a vital evidence gap and be beneficial in advancing treatment for the prevention of PTB.

Via bioinformatic analysis of RNA-sequencing (RNA-seq), PGR Chromatin Immunoprecipitation-sequencing (ChIP-seq), ESR1 ChIP-seq, and histone modification ChIP-seq using human myometrial tissues from pregnant women at term who were not in labor (TNIL) and women at term who were in labor (TIL), I discovered differentially expressed genes that were bound differentially by ESR1, PGR, and histone modifications, H3K4me3 and H3K27ac. Gene Ontology analysis uncovered that genes found highly expressed in TIL were highly enriched for pathways associated with acute inflammatory response and positive regulation of cytokine-mediated signaling pathways, whereas genes found highly expressed in TNIL were highly enriched for pathways related to muscle structure, muscle contraction, and cell junction assembly. These transcriptional differences led to distinct clustering between TIL and TNIL samples when

subjected to Principal Component Analysis. Because signal transduction leads to transcriptional changes, these transcriptional differences between labor status led to ChIP-seq experiments with two crucial pregnancy steroid receptors, ESR1 and PGR. ChIP-seq revealed that genomic regions differentially occupied by each transcription factor between TIL and TNIL were enriched in pathways like those identified in RNA-seq analysis, suggesting that ESR1 and PGR, via interaction with genomic loci, directly mediate the expression of genes associated with labor status. Integrative analysis of RNA-seq and ChIP-seq data of hormone receptors and histone marks uncovered ESR1/PGR downstream target genes potentially regulating labor status.

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List of Commonly Used Abbreviations

cAMP	Cyclic Adenosine Monophosphate
CAP	Contraction Associated Protein
ChIP-seq	Chromatin Immunoprecipitation Sequencing
COX	Cyclooxygenase
DEG	Differentially Expressed Gene
ESR1	Estrogen Receptor a
GJA1	Connexin 43
IP_3	Inositol 1,4,5-triphosphate
LPS	Lipopolysaccharide
MLCK	Mysoin Light Chain Kinase
MLCP	Myosin Light Chain Phosphatase
NP	Not Pregnant
OXTR	Oxytocin Receptor
PG	Prostaglandin
PGE	Prostaglandin E
PGR	Progesterone Receptor
PTB	Preterm Birth
PTGFR	Prostaglandin F Receptor
PTL	Preterm Labor
RLC	Regulatory Light Chain
RNA-seq	RNA Sequencing
RT-qPCR	Real-time Quantitative Polymerase Chain Reaction
TIL	Term in Labor
TNIL	Term Not in Labor

Chapter 1 INTRODUCTION TO PROGESTERONE AND ESTROGEN RECEPTOR ACTION IN HUMAN FEMALE PARTURITION

1.1 Introduction to Parturition

Parturition is the physiological process by which the fetus is expulsed from the uterus. Parturition, also called labor, involves strong and painful uterine contractions that push the fetus from the uterus. As labor progresses, the strong uterine contractile forces increase in frequency, duration, and intensity. Physiological changes such as cervical effacement, cervical dilation, and myometrial contractility are required steps for the onset of labor. Labor is a multifaceted phenomenon that can be seen as a feed-forward process involving the cervix, decidua, and myometrium, though this work focuses on the involvement of the myometrium.

1.1.2 Phases of Parturition

The biochemical changes of the uterus, critical for parturition, can be broken apart into four overlapping phases that occur throughout pregnancy (Figure 1-1). The first phase is known as uterine quiescence, in which the cervix is structurally sound and uterine smooth muscle cells remain in a non-contractile state during most of pregnancy. The myometrium undergoes slight periods of contractility before labor onset, known as Braxton Hicks contractions, that tone the uterus for labor. The second phase is uterine awakening, the phase in which myometrial changes are initiated to prepare the uterus for contractility. Awakening develops through the expression of the contraction-associated proteins (CAPs): oxytocin receptor (OXTR), prostaglandin F receptor (PTGFR), cyclooxygenase 2 (COX2), and connexin 43 (GJA1). These proteins play different roles in myometrial activation. Responsiveness to the hormone oxytocin is essential for contractility, and this is evidenced clinically by labor induction with synthetic oxytocin, Pitocin, and induced expression of the OXTR results in increasing the sensitivity to oxytocin required for labor (Maggi et al., 1990). Prostaglandins (PGs), specifically prostaglandin E2 (PGE_2) and prostaglandin F2-α

(PGF2α), are the main prostanoids administered clinically for cervical ripening and myometrial contractions (Giannoulius et al., 2002; Giannoulius et al., 2002; Hurd et al., 2008; Olson 2003). Increased expression of *COX2*, an enzyme that catalyzes PGs' formation, and PGF2α receptor (PTGFR) are important to mediate the uterotonic effects of these hormones. Gap junctions, such as the protein encoded by *GJA1*, in the myometrium are critical for electrical coupling between the smooth muscle cells during excitability to mediate cell-cell communication.

The third phase of parturition is labor, which can further be stratified into three stages: early/active labor, fetal delivery, and placental delivery. During the first stages of early/active labor, irregular myometrial contractions aid in cervical effacement and dilation, opening the cervical canal through the expulsion of the mucus plug. Upon cervical dilation of greater than six centimeters, myometrial contractile intensity will increase, and the frequency will become closer together, urging the fetus to descend the birth canal with the outcome of fetal delivery. The third and final stage of labor is the delivery of the placenta. The resulting tension from the emptying of the uterus and continual myometrial contractility separates the placenta from the implantation site, delivering the placenta through the birth canal.

The fourth and final phase of parturition is puerperium, the stage in which the reproductive organs return to their non-pregnant state. This phase is imperative to prevent infection of the reproductive tract and return to a state of fertility. Within four to six weeks following fetal delivery, the female reproductive tract undergoes uterine involution, the shrinking of the size of the uterus, and cervical remodeling and repair.

Phase 1	**Phase 2**	**Phase 3**	**Phase 4**
Uterine Quiescence	Uterine Awakening	Labor	Puerperium
Absence of uterine contractions	*Uterus is primed for labor*	*Uterine contractions, cervical effacement, and cervical dilation*	*Uterine involution, cervical repair, parturition recovery*

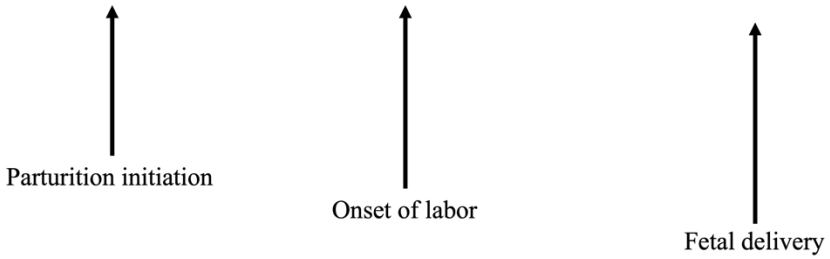


Figure 1-1

Phases of Parturition

1.2 Preterm Labor

The timing of labor is crucial for the survival of neonates. “Term” labor is defined as greater than 37 weeks of gestation, whereas “preterm” or “premature” labor is defined as less than 37 weeks of gestation. An estimated 15 million babies are born preterm annually across the world (WHO 2018), with approximately 1 million worldwide deaths from preterm birth (PTB) complications (Liu et al., 2016). In the United States, PTB is the leading cause of neonatal deaths (Martin et al., 2021). Surviving premature infants have a significantly increased risk of developmental delays, such as language, cognitive, sensory, and motor deficits (Hee Chung et al., 2020).

Racial disparities are present in PTB rates, with African American women exhibiting 4-5% increased PTB rates compared to non-Hispanic white women and Hispanic women in 2020 and over the past four years (Figure 1-2; National Vital Stats Report, 2020). The racial disparities seen in PTB rates may result from genetic, socioeconomic, environmental, obstetric, or other factors.

There is debate in the field on whether PTB and term labor share the same central process and signaling pathways leading to fetal delivery, despite gestational age. The central process, can be argued, as being shared due to the common physiological changes associated with the four stages of parturition, with PTB occurring earlier in gestation. Others in the field postulate that there is an entirely different process that results in PTB, with inflammation and other immunologically mediated pathways playing a significant role in increased risk for this defunct process (Romero et al., 2014; Cappelletti et al., 2016; Goncalves et al., 2002; Gomez et al., 1995; Kim et al., 2012; Elovitz et al., 2003; Phung et al., 2022). Although these two risk factors and other risks and biomarkers are reported and monitored, the exact etiology, or etiologies, of PTB is not yet clearly

defined (Lockwood et al., 1991; Inglis et al., 1994; Block et al., 1984; McGregor et al., 1995; Figure 1-3).

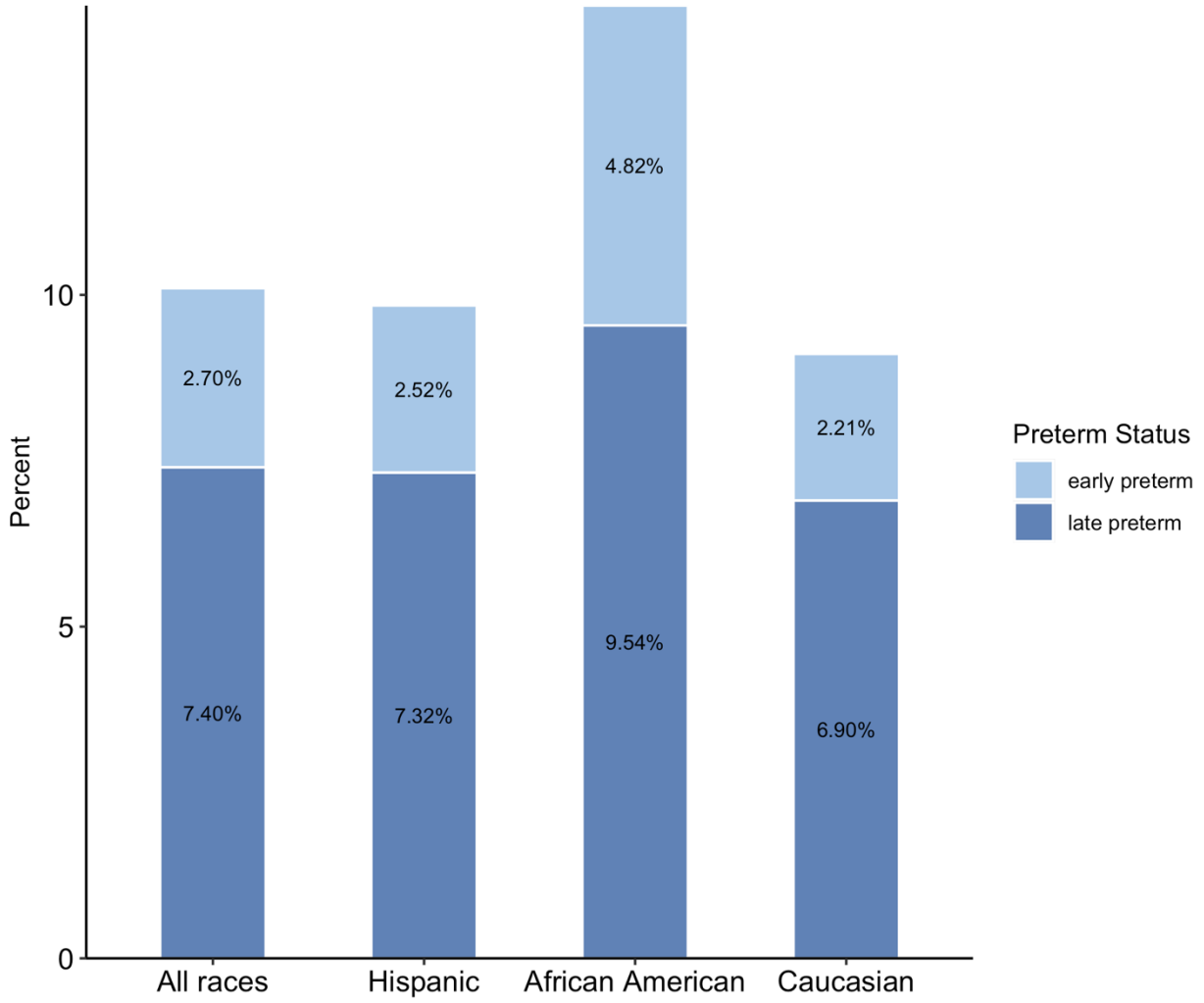


Figure 1-2

Percentage of preterm births, by race and Hispanic origin of mother in the United States for 2020. All non-Hispanic women are classified by race. Based on obstetric estimates, early preterm (under 34 weeks) and late preterm (34-36 weeks). Adapted from National Vital Statistics Report 2020

Marker	Diagnostic Specimen
Fibronectin	Cervicovaginal
Cytokines	Serum
Estradiol-17β	Serum
Estriol	Saliva
Progesterone	Serum

Figure 1-3

Table of biomarkers for the diagnosis of preterm labor

1.2.2 Tocolysis

Tocolysis is an obstetrical procedure using medications to delay spontaneous preterm labor (PTL) for two to seven days. Current drugs used clinically for tocolysis are beta-mimetics, calcium channel blockers, magnesium sulfates, nonsteroidal anti-inflammatory drugs, oxytocin inhibitors, and progesterone analogs. Each class has its own adverse maternal effects (Figure 1-4). The current goal of tocolysis is not to prolong gestation to full term but to delay PTL long enough for further interventions and treatments, such as antenatal corticosteroids to aid in fetal lung maturity, that increase the likelihood of positive fetal and maternal outcomes. Previous studies have found that the use of tocolytics in the maintenance of gestation is ineffective (Papatsonis et al., 2013; Naik Gaunekar et al., 2013; Han et al., 2013; Chawanpaiboon et al., 2014; Dodd et al., 2012).

Beta-mimetic drugs are the most used tocolytics in the U.S. (Mayer et al., 2022). Beta-adrenergic agonists increase intracellular cyclic adenosine monophosphate (cAMP) levels which inactivate the myosin light chain kinase, thus inhibiting myometrial contractility. The drug terbutaline can delay labor for up to 48 hours and has been used to treat PTL as early as 1990 (Lam et al., 1998). Although, due to the long-term effects on maternal health, neonatal outcome, and ineffectiveness, the U.S. Food and Drug Administration (FDA) has advised against treatment with terbutaline and recommends its use as a last resort in injectable form which should not be used in prolonged (beyond 48-72 hours) treatment for PTL since 2011 (Mayer et al., 2022).

Calcium (Ca^{2+}) signaling is crucial for smooth muscle contraction. Thus Ca^{2+} channel blockers that inhibit the Ca^{2+} influx are another option for tocolysis. Nifedipine, a calcium channel blocker, is a potent tocolysis with better neonatal outcomes and fewer maternal side effects than beta-mimetics (Hanley et al., 2019; Flenady et al., 2014). Magnesium sulfates were first reported in the clinical treatment of PTL in 1977 but have been studied on the effects of myometrial

contractility since the late 1950s (Steer et al., 1977; Mercer et al., 2009). Magnesium is postulated to affect myometrial contractility by blocking the influx of extracellular Ca^{2+} into channels and the release of intracellular Ca^{2+} via inositol 1,4,5-triphosphate (IP_3) channels (Fomin et al., 2006; Phillippe 1998).

PG inhibitors such as nonsteroidal anti-inflammatory drugs (NSAIDs) work through inhibition of COX that catalyzes PG production from arachidonic acid. Oxytocin inhibitors, specifically Atosiban, were developed as a tocolytic but are not currently approved by the FDA for use in the U.S. (Akerlund et al., 1985; American College of O and B., 2016). Oxytocin stimulates myometrial contractions by increasing the intracellular levels of IP_3. Atosiban acts as an oxytocin receptor antagonist in addition to a vasopressin receptor antagonist, which impacts PGE signaling (Kim et al., 2019).

Hydroxyprogesterone caproate has been studied since the 1970s as a treatment for PTL. Treatment with hydroxyprogesterone caproate was associated with a significant elongation of gestation (Johnson et al., 1975). Treatment with progesterone vaginally and intramuscularly is associated with reducing spontaneous PTL in women (Fonseca et al., 2007; Maher et al., 2013). Furthermore, a meta-analysis of progesterone use in the treatment of PTL has concluded that overall, progesterone reduces the rates of this syndrome (Rode et al., 2009; E. Group, 2021).

Despite tocolysis procedures, the rate of PTB in the U.S. has insignificantly decreased for the past four years (National Vital Stats Report, 2020). Thus, there is a critical need for a better understanding of the pathobiology of PTB to develop proper preventative therapeutic strategies for this syndrome.

Drug	Mechanism of Action	Maternal Adverse Effects
Terbutaline (Bricanyl, Marex)	Beta-2 adrenergic agonist	Shakiness, tremors, tachycardia, shortness of breath, pulmonary edema
Nifedipine (Procardia, Adalat, Afeditab)	Calcium channel blocker	Dizziness, hypotension, flushing, headache
Aspirin	NSAID, COX inhibitor	Nausea, gastritis, esophageal reflux
Indomethacin	NSAID, COX inhibitor	Nausea, gastritis
Atosiban (Tractocile, Antocin)	Oxytocin inhibitor	Nausea, vomiting, headache
Hydroxyprogesterone caproate (Makena)	Progesterone analog	Bloating, nausea, headache

Figure 1-4

Table of current tocolytic agents, their mechanism of action, and reported adverse effects in the treatment of PTL

1.3 Uterine Contractility

Many pathways are mechanistically involved in uterine smooth muscle cell contractility, and the intracellular and extracellular concentrations of ions determine the intensity of myometrial contractions (Hutchings et al., 2009). The process of smooth muscle stimulation is a bioelectric process requiring action potentials involving ions. First, the muscle must start in the resting phase, which involves the bi-directional movement of potassium (K^+) ions in and out of the cell and the stasis of sodium (Na^+) ions outside the cell membrane.

Like most smooth muscle cells, myometrial smooth muscle cell contractions are mediated by calcium signaling (Figure 1-5). Following electrical muscle excitation, Ca^{2+} is released from the sarcoplasmic reticulum and moved into the cell via voltage-gated L-type channels. Changes to intracellular calcium are integral in smooth muscle contraction through binding with Calmodulin. This complex results in the phosphorylation and, therefore, activation of the myosin light chain kinase. Following activation, this kinase will phosphorylate myosin's regulatory light chain (RLC). Myosin consists of two heavy chains that coil around each other, forming a double helix body, and four light chains, two of which are RLCs while the others are essential light chains (ELCs), that are in the head of myosin (Craig and Woodhead, 2006; Rayment et al., 1993). Activated myosin will bind to its partner, actin, and these proteins bring about muscle shortening (Figure 1-6).

Gap junctions are gaps between cells composed of connexin proteins. Myometrial cells are coupled together by gap junctions, fundamental components of myometrial contractility, mediating cell-cell communication (Garfield et al., 1988; Garfield et al., 1995). When action potentials occur in the smooth muscle cells, the potential will propagate down the gap junctions, bringing contractility to all the smooth muscle cells, resulting in muscle fiber contraction.

In contrast, relaxation of smooth muscle cells is mediated not only by decreases in Ca2+ levels but by cAMP and cyclic guanosine monophosphate (cGMP). Nitric oxide (NO) increases cGMP levels, which leads to activation of the myosin light chain phosphatase (MLCP), which dephosphorylates the myosin light chain, resulting in no cross-bridge movement and thus smooth muscle relaxation. Additionally, tocolytic substances inhibit muscle contraction, such as beta-adrenergic receptor agonists, by inhibiting MLCK (Figure 1-5).

1.4 Steroid Hormone Role in Myometrial Contractility and Parturition

1.4.2 Progesterone and Progesterone Receptors

Progesterone plays a crucial role in the establishment and maintenance of pregnancy. Progesterone *in vitro* and *in vivo* decreases myometrial contractility and inhibits myometrial gap junction formation in animal models and humans (Garfield et al., 1998; Da Fonseca et al., 2003). Many mammals show a decline in circulating progesterone levels proceeding labor initiation (Young et al., 2001). This decline in serum progesterone led to the hypothesis that the abatement of progesterone initiates the onset of labor. However, circulating progesterone levels remain high in humans during gestation (Boroditsky et al., 1978; Tulchinsky et al., 1972; Walsh et al., 1984). This led to a "progesterone block" theory whereby progesterone is no longer functional, through an unknown threshold mechanism where progesterone with uterotonics, such as estrogen, PGs, and oxytocin, to maintain the delicate balance to maintain pregnancy (Csapo and Pinto-Dantas, 1965; Figure 1-7). However, recently there has been evidence of physiological withdrawal of progesterone, but not receptors, at the myometrial level supporting a functional progesterone withdrawal theory in which myometrial progesterone responsiveness is decreased (Nadeem et al., 2016).

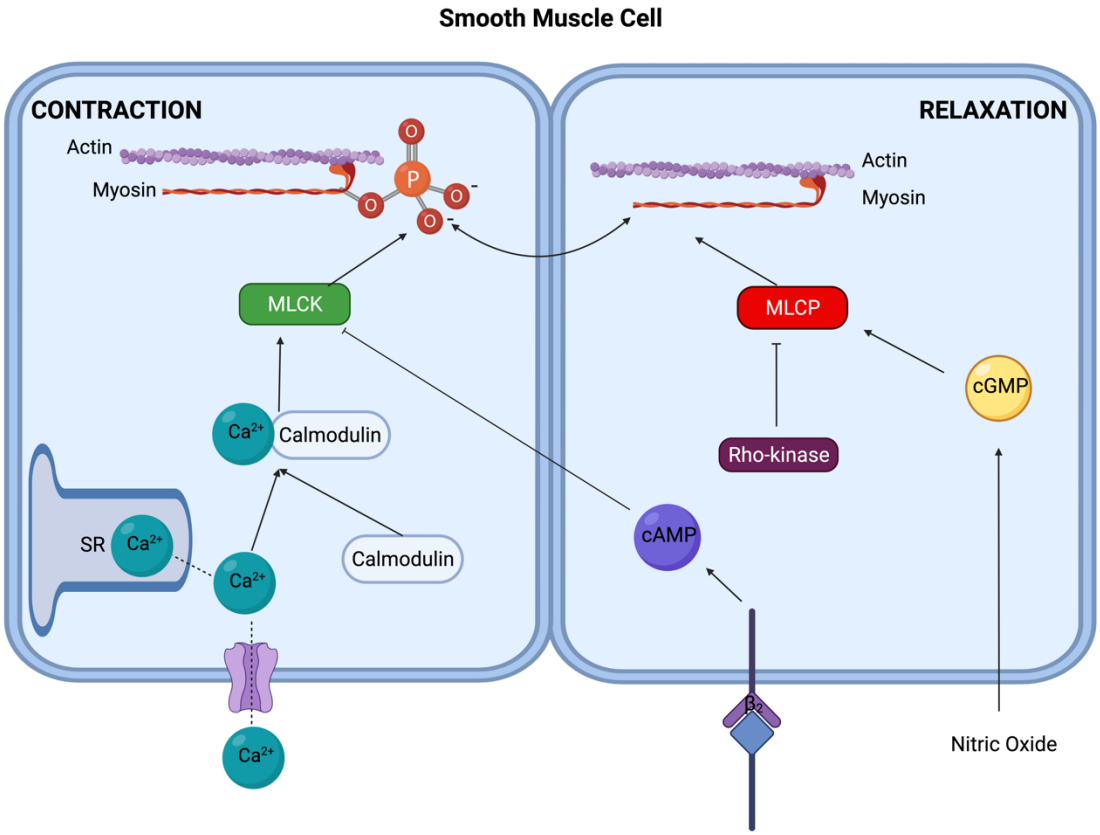


Figure 1-5

The smooth muscle contraction pathway is mediated by calcium, and changes in cAMP and cGMP mediate smooth muscle relaxation. Created with BioRender.com

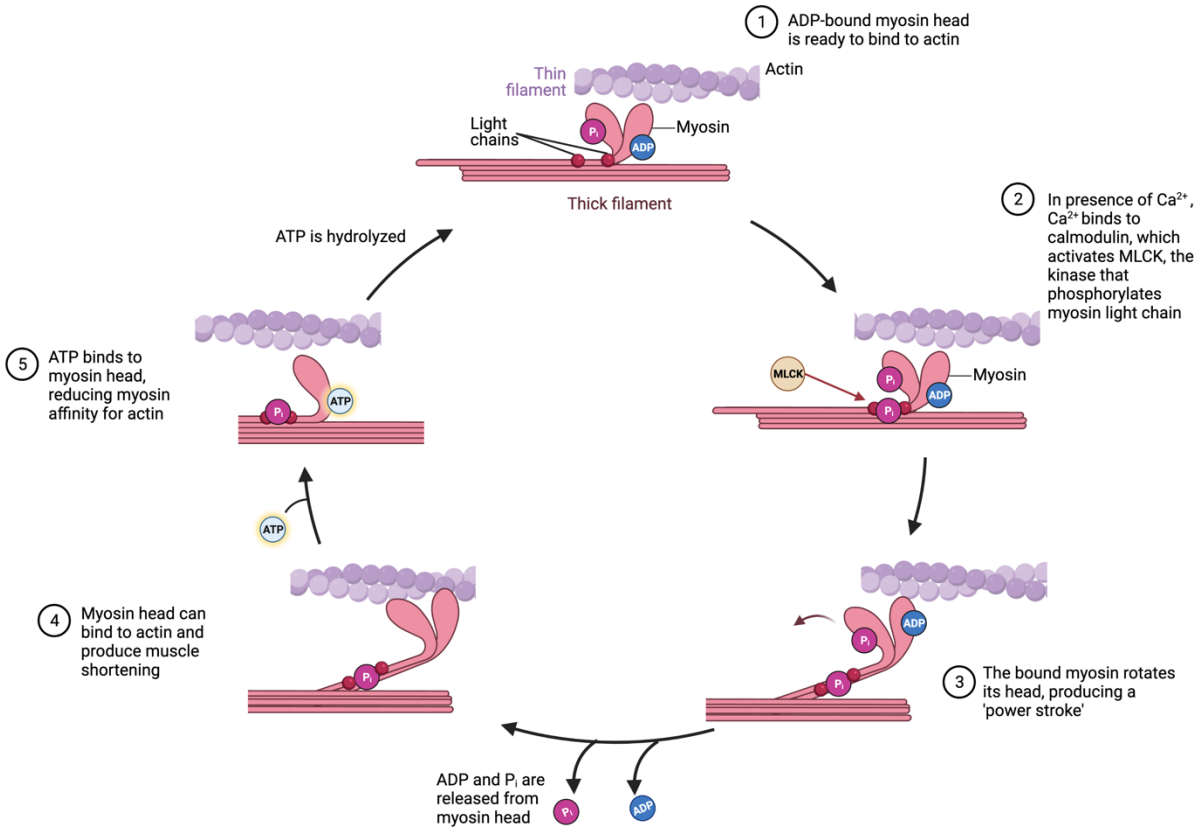


Figure 1-6

The muscle shortening process is brought upon by myosin and actin cross-bridges through the phosphorylation of regulatory light chains of myosin. Created with BioRender.com

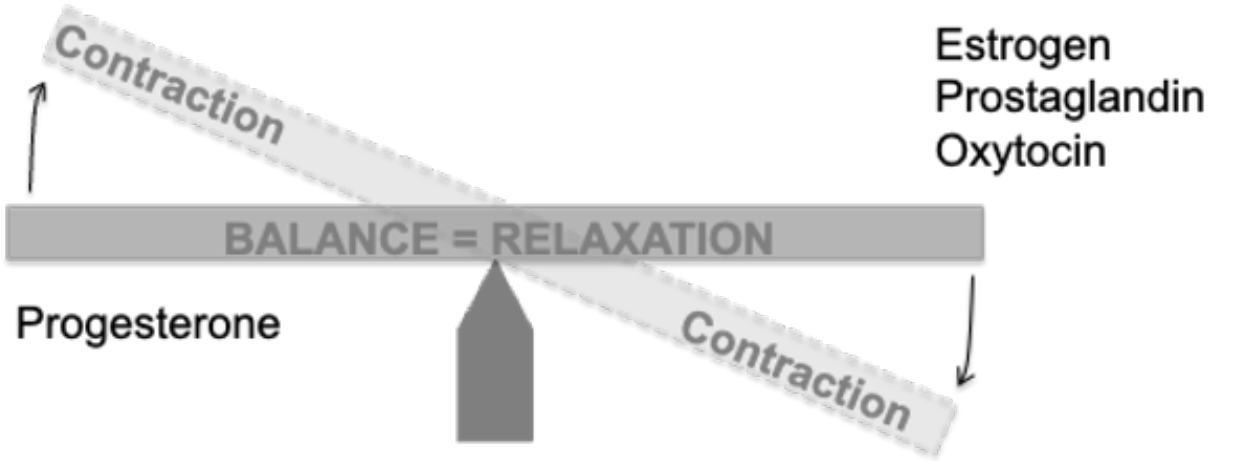


Figure 1-7

The delicate balance between progesterone and uterotonics in maintaining myometrial relaxation or myometrial contractility

Additional evidence of a functional progesterone withdrawal theory in the human labor is demonstrated by spontaneous pregnancy loss when mothers are treated with a progesterone receptor (PGR) antagonist, mifepristone (RU486), at any stage of pregnancy (Avrech et al., 1991; Chwalisz et al., 1991; Chwalisz et al., 1994; Neilson et al., 2000). Other groups have tested the hypothesis that changes in myometrial PGR isoform expression mediate functional progesterone withdrawal. The human *PGR* gene encodes two major products, the full-length PGR-B and the N-terminus truncated PGR-A, under two distinct promoters (Chauchereau et al., 1992; Bouchard et al., 1999; Giangrande et al., 1999; Conneely et al., 2000). Increased mRNA and protein expression of PGR-A versus PGR-B were found in term pregnant myometrial biopsies. It is now widely accepted in the field that spontaneous onset of labor is preceded by dynamic changes in PGR isoform ratio with PGR-B dominance earlier in pregnancy and PGR-A dominance preceding spontaneous labor (Merlino et al., 2007; Pieber et al., 2001; Haluska et al., 2002; Mesiano et al., 2002).

The physiological relevance of changes in the ratio of PGR isoforms has been studied in myometrial cells. It was found that increases in PGR-A/PGR-B decrease PGR-B mediated progesterone responsiveness, possibly playing a role in functional progesterone withdrawal (Merlino et al., 2007; Pieber et al., 2001). Functional progesterone withdrawal can also be mediated by inhibition of PGR DNA binding. A decline in PGR co-activators, cAMP response element binding protein (CREB)-binding protein (CBP), and steroid receptor coactivators-2 and 3 (SRC2 and SRC3) was associated with labor in human myometrium (Condon et al., 2003). In addition, differences in affinity for activator protein 1 (AP-1) and co-repressors that regulate the expression of *GJA1* in the myometrium may also contribute to functional progesterone withdrawal.

PGR-B was shown to have a more robust interaction with AP-1 proteins, cJun and JunB, and co-repressors $p54^{nrb}$ and mSIN3A (Nadeem et al., 2016).

Another study identified a protein known as polypyrimidine tract-binding protein-associated splicing factor (PSF), which interacts with PGR and acts as an inhibitor of PGR transcriptional activity in mammalian cells (Dong et al., 2005). Additionally, the same study demonstrated in pregnant rat tissues that a significant increase in *PSF* myometrial expression was associated with reduced PGR protein expression. Interestingly, PSF regulates the splicing, and thereby activity, of MLCP, a key dephosphorylator/inactivator of the myosin light chain. Thus, PSF functions in the relaxation of smooth muscle cells (Figure 1-5).

1.4.3 Estrogen and Estrogen Receptors

The steroid hormone estradiol and its receptors have been found to play an essential role in pregnancy and labor. During pregnancy, estradiol levels are drastically increased compared to before pregnancy. Estriol levels consistently rise 2-4 weeks before the onset of labor, suggesting the importance of estrogen in labor (Boroditsky et al., 1978). The roles of estrogens in human pregnancy were confirmed back in 1967 when non-laboring pregnant women were administered 200 mg 17□-estradiol intravenously. Estradiol treatment increased uterine contractility and responsiveness to oxytocin treatment within 4-6 hours. As a result, quickened time to delivery was also observed. The CAPs: COX2, OXTR, and GJA1 are induced by estrogen and increase dramatically prior to the onset of labor in human myometrium and animal models (Jackson, 1998; Kilarski et al., 1996; Kilarsaki et al., 2000; Nissenson et al., 1978; Petrocelli and Lye, 1993). In rabbits, estrogen treatment was associated with increased myosin light chain kinase and calmodulin activity and content, respectively (Matsui et al., 1983).

Estrogen receptor alpha (ESR1) is found to be increased in laboring myometrium compared to non-laboring myometrium and is the dominant estrogen receptor isoform in pregnant myometrium (Mesiano et al., 2002; Welsh et al., 2012; Mesiano and Welsh, 2007), suggesting that ESR1 promotes contractility and excitability of the myometrium. COX2 is the gene that encodes an enzyme important to producing PGs, which triggers the release of oxytocin from the pituitary gland. Increases in oxytocin levels do not coincide with the onset of labor. However, increases in OXTR levels and density of OXTR have been observed in the myometrium of pregnant women at the onset of labor (Kimura, 1996). Increases in *COX2* and *OXTR* mRNA are correlated with increased expression of ESR1, suggesting they are ESR1 target genes (Smith, 2002).

1.4.4 Progesterone/Estrogen Interaction

In pregnancy, there is a dynamic balance between progesterone and estrogen in the control of uterine activity (Figure 1-7). With the drastically increased estrogen levels throughout pregnancy, the actual mechanism of ESR1 regulation of the onset of labor at term rather than earlier in gestation has not been elucidated. A positive correlation between mRNA levels of ESR1 and the PGR-A: PGR-B ratio at term was found in non-laboring human myometrium, and increases in the PGR-A: PGR-B ratio and ESR1 mRNA levels were observed in term laboring myometrium (Mesiano et al., 2002). A positive correlation was also found between ESR1 mRNA levels and CAPs in non-laboring myometrium, further supporting uterine ESR1 in activating CAP expression and uterine contractility. Additional studies have shown the association of progesterone levels and PGR isoforms with the suppression of ESR1 expression/activity and uterine estrogen responsiveness in animal models (Katzenellenbogen et al., 2000; Leavitt et al., 1987; Okulicz et al., 1981; Okulicz et al., 1981; Okulicz et al., 1981). Taken together, these results suggest that

progesterone and PGR isoforms may play a role in modulating the expression of ESR1 in the myometrium leading to its specificity in the regulation of CAPs at term.

1.5 Animal Models

Many studies have aimed to provide mechanistic insight into the labor cascade. Most studies to date have focused on using animal models or human myometrial cell models to study parturition-associated pathways. Animal models are essential tools for investigating proteins, gene expression, and pathways involved in various diseases and conditions. Consequently, many groups have developed different animal models to study parturition and preterm labor. Current animal models of labor include mice, rats, rabbits, sheep, and rhesus monkeys. One drawback of a portion of current animal models is the mechanism of labor, allowing for limited insight. To understand the mechanism of labor and preterm labor in humans without the use of human subjects, the pathobiology of parturition in some animal models is modulated or altered to mimic that of humans. In mice, rats, rabbits, and sheep, labor at term occurs after a drop in serum progesterone, opposite of what is observed in humans and non-human primates (Garfield et al., 1998; Da Fonseca et al., 2003; Young et al., 2001; Challis et al., 2000).

Despite this difference, rodents are a cost-effective model for studying parturition on top of the ease and feasibility of producing large numbers of progeny for future studies concomitantly. Additionally, mice are great models for genetic manipulation, in which knockout models can be utilized to differentiate important pathways involved in labor. Manipulations in neuropeptides, prostaglandin enzymes, prostaglandin receptors, steroid receptor co-activators, and more have been utilized (Muglia, 2000; McCarthy et al., 2018; Roizen et al., 2019). Preterm labor rodent models have been established via intrauterine treatment with lipopolysaccharides (LPS) or

cytokines and are frequently used in the field (Kaga et al., 1996; Romero et al., 1991; Elovitz et al., 2003; Bennett et al., 2000). It is argued that these animal models mimic inflammation-induced preterm labor in humans. Still, one caveat is that this model does not address vaginal or cervical inflammatory responses that may initiate preterm labor.

Rabbits are another animal in which preterm labor can be provoked by intra-amniotic injection of cytokines (Bry and Hallman 1993). The original progesterone withdrawal theory was supported by a rabbit model (Csapo 1956). Furthermore, drug-induced progesterone withdrawal can cause earlier labor in rabbits treated concomitantly with antiprogestins and oxytocin compared to control and oxytocin (Bernard et al., 1980). Although these animals were used extensively in the beginning studies of parturition and preterm labor, they are not often used to date. Sheep are great models for studying uterine activity. Opposite to the rodent and rabbit models, intra-amniotic treatment of LPS in sheep does not induce preterm labor but increases uterine activity (Grigsby et al., 2003). A disadvantage of this animal model is the expense and restrictions on the numbers of these animals for experimental studies.

The ideal animal model for human labor would be non-human primates, as they demonstrate similar reproductive biology, mechanism of labor, and uterine activity can also be studied (Mecenas et al., 1996). Intra-amniotic infusion of LPS in the monkey can increase the contractile activity of the uterus and induce preterm birth (Gravett et al., 1994; Gravett et al., 1996). However, exorbitant costs of animal husbandry and restrictions associated with non-human primates limit their use for studies. Thus, rodent models, mainly mice, are the most widely used animal model for parturition and preterm labor.

1.6 Limitations of Current Studies

Although mice models are the most widely used *in vivo* model to investigate human parturition, cells are also useful for mechanistic studies of conditions and diseases. Primary cells are a valuable *in vitro* tool to gain insight into biological processes by providing a representation of the original tissue and phenotype of interest. Primary cells derived from the myometrium of non-pregnant and pregnant term women show contractile response to uterotonics and inflammatory signals (Dallot et al., 2003; Devost and Zingg, 2005; Fitzgibbon et al., 2009). Yet, the limited lifespan of cultured primary cells and their ability to continue representing their origin is a cause of concern for researchers. As a result, immortalized cell lines have been developed to address this issue (Perez-Reyes et al., 1992; Mosher et al., 2013; Condon et al., 2002).

The immortalized pregnant human myometrial (PHM1-41) cell line was derived from term pregnant myometrium and has been utilized for mechanistic parturition studies (Monga et al., 1996). These cells retain the same smooth muscle cell morphology as tissue, retain estrogen receptor expression, are responsive to oxytocin, and express GJA1 and smooth muscle actin (Monga et al., 1996; Burghardt et al., 1996; Burghardt et al., 1999). LPS-treated PHM1-41 cells secreted inflammatory cytokines, chemokines, and PGF2α (Hutchingson et al., 2013). Furthermore, inflammation also induced PHM1-41 contractility. In contrast, progesterone was found to inhibit cytokine-induced contractility of PHM1-41 cells (Rajagopal et al., 2015). Likewise, androgens inhibit PHM1-41 basal contractility (Makieva et al., 2016).

However, the most widely used myometrial cell line in parturition studies are human telomerase reverse transcriptase- myometrial (hTERT- HM) cells. Like their pregnant counterpart, these cells are morphologically like primary myometrial cells from term pregnant uterus, express typical myometrial cell line genes (*e.g., OXTR*, *GJA1*, *ESR1, PGR,* smooth muscle actin), and are

responsive to oxytocin (Condon et al., 2002; Renthal et al., 2010). Despite the similarities, there are striking differences from *in vivo* conditions, such as diminished progesterone responsiveness, low PGR mRNA expression, and nearly undetectable PGR-A and PGR-B protein levels (Merlino et al., 2007). Thus, many researchers have engineered these cells to modulate the levels of PGR-A and PGR-B in the hopes of mimicking *in vivo* PGR-A: PGR-B levels, making them ideal for studying the role of progesterone in augmenting pathways associated with preterm and term myometrium to dispute or support the functional progesterone withdrawal theory. This excessively used cell line, although useful, cannot fully mimic the myometrial environment of preterm or term pregnant tissues.

The goal of most studies in the field are to expand our understanding of the underlying mechanisms hormones play in parturition to develop better therapeutics for preterm labor prevention. Yet, further information is required to investigate the genomic roles of progesterone receptor and estrogen receptor for the switch of quiescent myometrium to contractile myometrium in labor. In the current dissertation, the role of progesterone and estrogen receptors in the induction of labor is explored using human myometrial tissues from term pregnant women in labor and not in labor. I hypothesize that progesterone and estrogen receptors play a role in regulating the transcriptome of human myometrium that defines labor status with progesterone receptor playing a role in inducing and inhibiting smooth muscle contraction associated genes responsible for labor.

Chapter 2. GENOME-WIDE TRANSCRIPTIONAL CHANGES IN PREGNANT MYOMETRIAL TISSUES REVEAL PATHWAYS THAT MAY HAVE IMPLICATION IN LABOR

2.1 Introduction

Parturition is a process that involves cervical ripening, decidual activation, and myometrial activation (Alfaidy et al., 2003; Brown et al., 2006; Carvajal et al., 2006; Challis et al., 1986; Challis et al., 1999; Challis et al., 2000; Challis et al., 2005; Chaudhari et al., 2008; Gibb et al., 2002; Imamura et al., 2000; Lei et al., 1996; Lei et al., 1999; Norwitz et al., 1999; Olson et al., 1995; Romero et al., 2006; Tornblom et al., 2004; Xu et al., 2002). During this process, the myometrium transitions from a quiescent state to a contractile state (Bytautiene et al., 2004; Carbillon et al., 2001; Cong et al., 2009; Cordeaux et al., 2009; Dalrymple et al., 2007; de Wit et al., 2010; Fischer et al., 2008; Grammatopoulos et al., 2007; Patel et al., 2001). It is postulated that gene expression changes are responsible for the change in myometrial cell phenotype. Thus, gene expression studies have been conducted on term laboring, non-laboring, and preterm human tissues. The earliest studies investigated specific gene sets or performed functional genomics (Aguan and Carvajal, 2000; Chan et al., 2002; Esplin et al., 2005). Others performed microarray and RNA-sequencing (RNA-seq) (Bethin et al., 2003; Bukowski et al., 2006; Chan et al., 2014; Esplin et al., 2005; Havelock et al., 2005; Mittal et al., 2011, Mittal et al., 2010; O'Brien et al., 2008; Sharp et al., 2016). These studies identified a plethora of differentially expressed genes between laboring and non-laboring myometrium, and many genes and pathways found are common across studies. Despite the aggregate information, a reliable transcriptional network for laboring human myometrium has not been identified (Eidem et al., 2015; Stanfield et al., 2019).

A recent meta-analysis of microarray and RNA-seq data uncovered that, on aggregate, studies show that inflammatory pathways, including expression of cytokines and chemokines, are highly associated with laboring myometrium, and pathways involved in smooth muscle relaxation and cell adhesion are associated with non-laboring myometrium (Stanfield et al., 2019). Yet, the

transcriptomic profiles found by RNA-seq were using a low number of reads, lower than the recommended protocol in the field, and all aligned to the older reference human genome, hg19. To have a higher resolution of the gene expression dynamics in the switch from quiescent myometrium to contractile myometrium, sequencing at a higher depth should be performed on laboring and non-laboring tissues and aligned to the most recent reference human genome, hg38. Thus, this study aims to address this issue and identify a more reliable transcriptional network of term in labor (TIL; >37 weeks with signs of labor) and term not in labor (TNIL; >37 weeks with no signs of labor) myometrium to further classify genes and pathways associated with parturition.

2.1 Results

Clinical Characteristics of Myometrial Specimens

The characteristics of the pregnant patients from whom the myometrial tissues were collected and used for RNA-seq experiments are described in Table 2-1. The RNA-seq experiments were conducted on 11 samples (TNIL, n=7; TIL, n=4). Results showed no statistical differences in maternal age, parity, body mass index (BMI), and gestational age among the groups. Our patients were racially diverse, consisting of African American and Caucasian women.

Distinct transcriptomes of TIL and TNIL myometrial tissues

To broaden our understanding of the dynamics between TIL and TNIL myometrial tissues, samples were subjected to RNA-seq for genome-wide transcriptomic profiling at a depth of 33 million reads. Overall transcriptomic profiles show distinct clustering by labor status as demonstrated by principal component analysis (PCA) (Figure 2-1), suggesting significant transcriptomic differences between TIL and TNIL myometrial tissues. Using a cutoff of a false discovery rate < 0.05, 1414 genes were found to be significantly differentially expressed (DEGs).

	Maternal age (years)	Parity	BMI (kg/m^2)	GA at delivery (weeks)
RNAseq				
TNIL (n=7)	33.14 ± 1.16	1.14 ± 0.46	31.33 ± 2.05	39.17 ± 0.08
TIL (n=4)	35.75 ± 2.56	0.75 ± 0.25	31.33 ± 2.76	39.25 ± 0.38
p-value	0.403	0.473	1.000	0.851

Data are expressed as mean ± SEM. TNIL, term not in labor; TIL, term in labor; BMI, body mass index; GA, gestational age. p-values are from ttests between TNIL and TIL for each -omic experiment.

Table 2-1

Clinical characteristics of myometrial specimens for RNA-seq

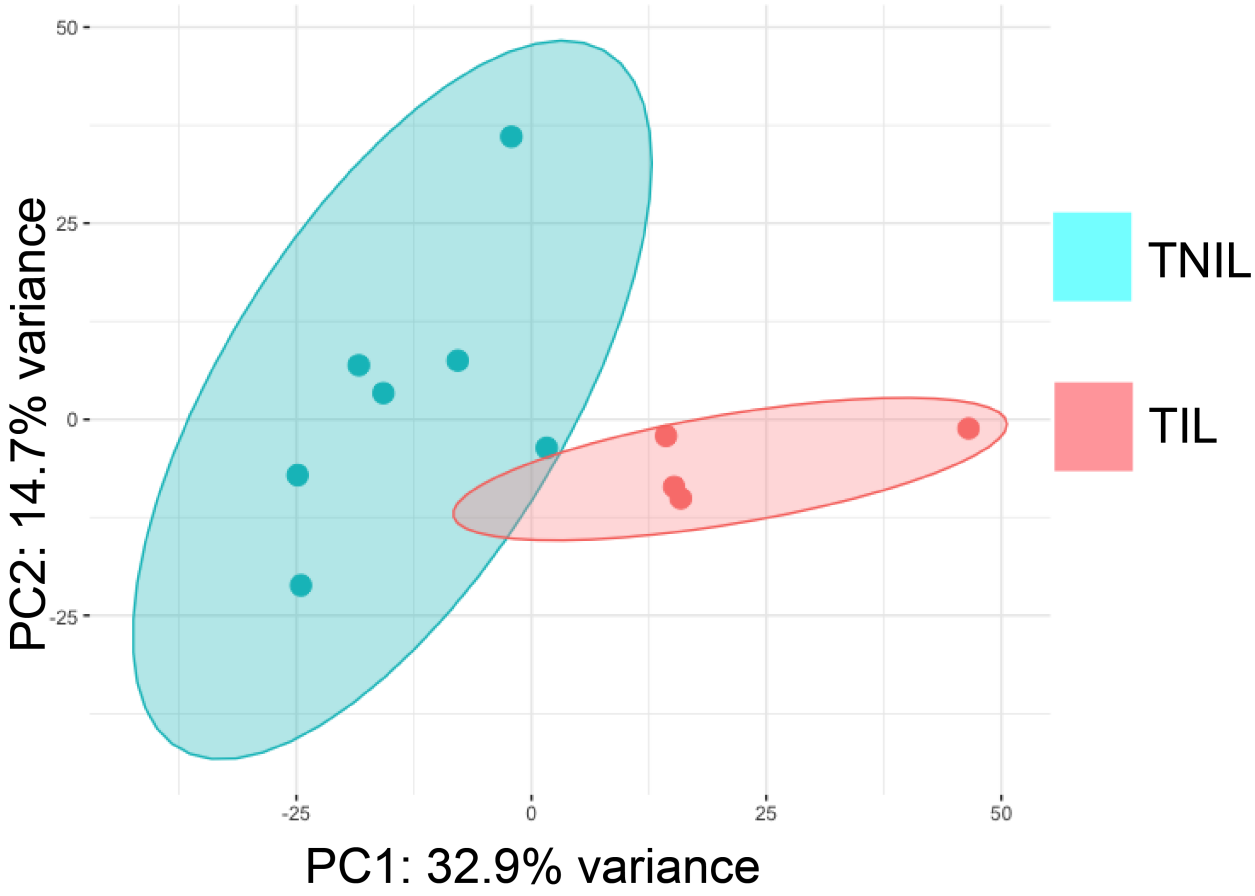


Figure 2-1

PCA of seven TNIL (blue) and four TIL (pink) samples show distinct clustering between the groups, demonstrating there are critical differences between laboring and non-laboring myometrium that contribute to the phenotype

Differential expression analysis uncovers genes enriched in

The expression of 605 genes was higher in TNIL and 809 genes higher in TIL tissues, coined downregulated and upregulated in labor, respectively (Figure 2-3). Among all the DEGs, the topmost upregulated gene in labor was Mucin 5B (MUC5B), a gel-forming mucin found in cervical mucus (Figure 2-2). The second most upregulated gene was progestagen-associated endometrial protein (PAEP), a secreted glycoprotein produced by the endometrium during pregnancy. Other top upregulated genes served various biological processes, such as ion transport, innate immunity, and ligand binding (Table 2-2). The topmost downregulated gene in labor was acrosomal protein (KIAA1210), followed by CUB and Sushi Multiple Domains 1 (CSMD1). Among the top downregulated genes, calcium-binding genes were highly prevalent, along with G protein-coupled receptor activity genes (Table 2-3). Parturition-related genes such as OXTR, GJA1, and ZEB1 were not found to be significantly different between labor statuses (Table 2-4).

Functional gene ontology of differentially expressed genes

Gene ontology analysis on the significant DEGs indicated that genes upregulated in labor are enriched in pathways associated with calcium signaling, inflammation, and cytokine signaling, consistent with labor associating with inflammatory response in the myometrium (Figure 2-4). When looking at the top 100 upregulated genes, 100% of the pathways are inflammatory and immune-related, further supporting the link between labor and inflammatory response (data not shown). In contrast, the top 100 downregulated genes were associated with cell adhesion, integrin signaling, and phosphorylation pathways (data not shown). In total, genes downregulated in labor are enriched in terms associated with muscle contraction, actin-filament based movement, cell-matrix adhesion, and integrin signaling (Figure 2-4). These findings suggest that the muscle contraction and actin movement enriched pathways found from the DEGs increased in TNIL may

be softening the uterus for labor. In contrast, these processes have already occurred in the TIL group.

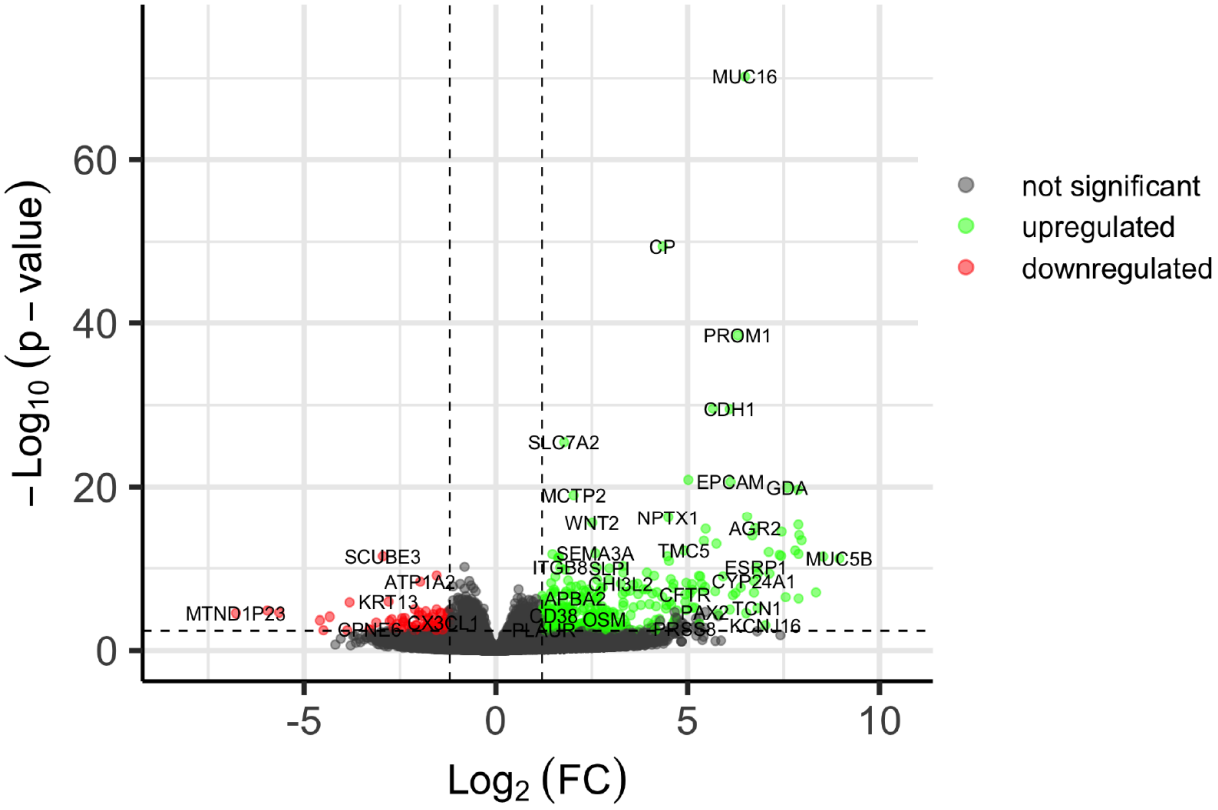


Figure 2-2

Volcano plot showing all detected transcripts with significant genes (FDR < 0.05) shown as upregulated (green) or downregulated (red) in TIL generated by DESeq2 analysis.

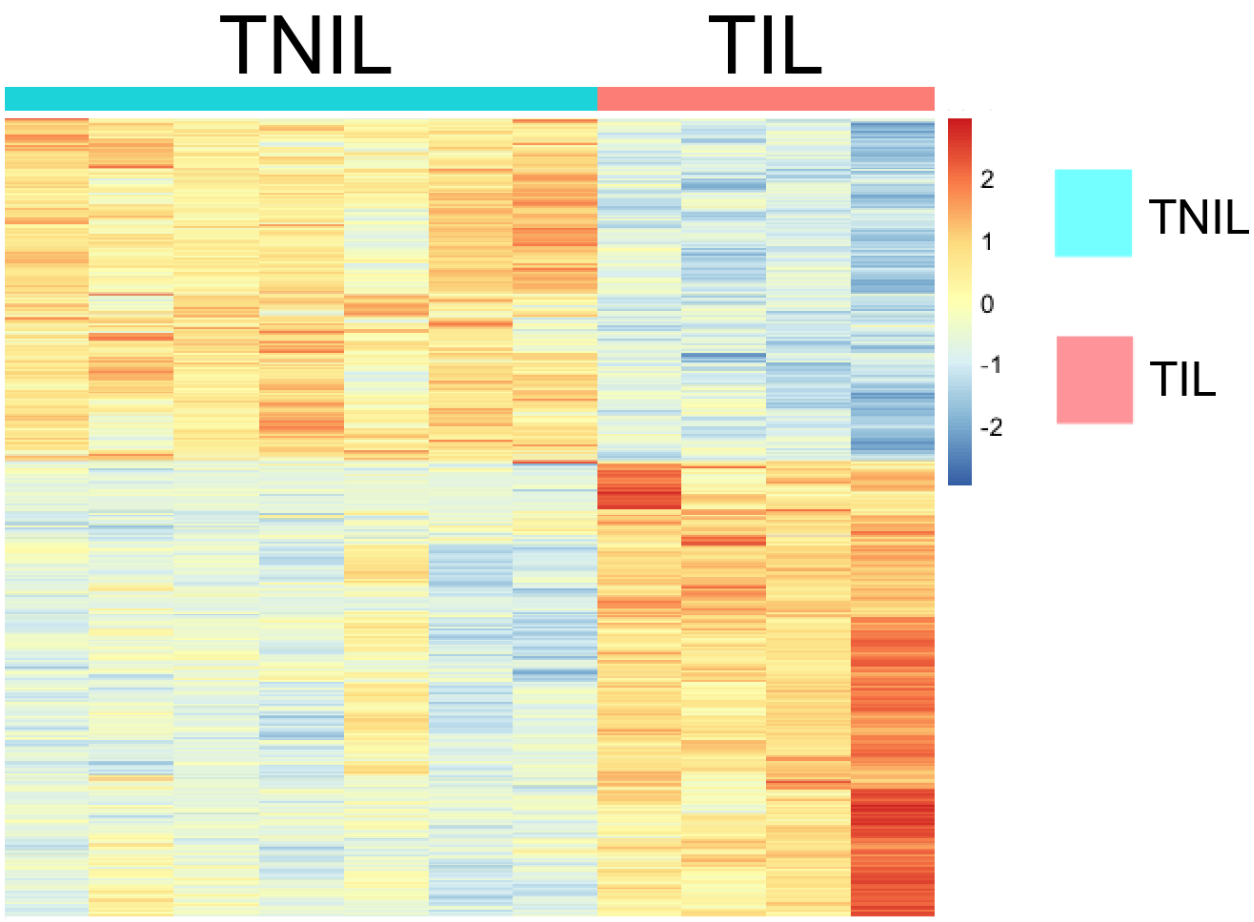


Figure 2-3

Heatmap of significant differentially expressed genes in seven TNIL (blue) and four TIL (pink). The colors represent the degree of up and downregulated genes, red and blue, respectively.

Gene Symbol	Fold Change	Log_2 Fold Change	p-value	adjusted p-value
MUC5B	495.400	8.952	6.05E-12	2.88E-09
PAEP	370.730	8.534	3.59E-12	1.86E-09
LINC01541	324.925	8.344	9.16E-08	1.60E-05
SCEL	250.605	7.969	3.64E-14	3.06E-11
LINC01502	239.768	7.905	8.55E-15	7.93E-12
LINC01317	237.388	7.891	5.06E-07	7.02E-05
SLC34A2	237.038	7.889	1.73E-12	1.09E-09
WDR72	236.587	7.886	4.52E-16	5.31E-13
PIGR	235.136	7.877	2.05E-20	3.61E-17
MUC6	223.136	7.802	6.51E-13	4.69E-10
GDA	193.769	7.598	1.14E-20	2.23E-17
UCA1	187.729	7.553	3.40E-07	5.00E-05
WNT7A	174.505	7.447	3.27E-15	3.20E-12
UGT2B7	172.999	7.435	2.97E-12	1.69E-09
HNF1B	168.026	7.393	2.27E-12	1.34E-09
SLC6A14	139.974	7.129	4.48E-10	1.61E-07
C2CD4A	138.431	7.113	9.81E-13	6.65E-10
KCNJ16	130.263	7.025	0.000813396	0.017761268
MMP10	113.721	6.829	4.23E-10	1.55E-07
TCN1	113.047	6.821	7.46E-06	0.000625858

Table 2-2

List of topmost upregulated genes in laboring myometrium show transporter genes, mucins, and metallopeptidases

Gene Symbol	Fold Change	Log_2 Fold Change	p-value	adjusted p-value
KIAA1210	0.071	-3.817	1.46E-06	0.000167463
CSMD1	0.101	-3.302	0.002885399	0.040612227
CPNE6	0.102	-3.294	0.002605617	0.038263479
TNNC2	0.115	-3.120	0.000373422	0.010379237
SCUBE3	0.130	-2.948	3.24E-12	1.75E-09
KRT13	0.144	-2.798	1.19E-06	0.000143828
TENM2	0.146	-2.772	0.000260097	0.008487817
ACTA1	0.148	-2.758	0.00388347	0.049056996
ERICH5	0.153	-2.713	0.000168745	0.006325613
LOC574538	0.156	-2.682	0.001725973	0.029454542
ARL17A	0.183	-2.447	0.00012556	0.005147593
MCHR1	0.188	-2.413	0.000358933	0.010201813
VIPR1	0.189	-2.407	0.000254719	0.00835878
CRB2	0.192	-2.381	0.001955594	0.032022287
GRIN2A	0.205	-2.288	0.000907595	0.019062743
SBSPON	0.219	-2.193	0.002931742	0.041035072
FDXR	0.231	-2.115	1.01E-05	0.000766957
LINC02610	0.232	-2.106	0.003090998	0.042224477
CEND1	0.236	-2.084	0.003487461	0.045864442
NTRK3	0.236	-2.082	0.000753683	0.016748308

Table 2-3

List of topmost downregulated genes in laboring myometrium

Gene Symbol	Fold Change	Log $_2$ Fold Change	p-value	adjusted p-value
OXTR	0.527	-0.925	0.047	0.204
GJA1	1.225	0.292	0.281	0.546
ZEB1	0.930	-0.104	0.560	0.772
PGR	1.316	0.397	0.145	0.385

Fold Change = Fold change of gene expression in labor

Log 2 Fold Change = Log 2 fold change of gene expression in labor

Table 2-4

List of labor-associated genes and their expression changes between laboring and non-laboring myometrium

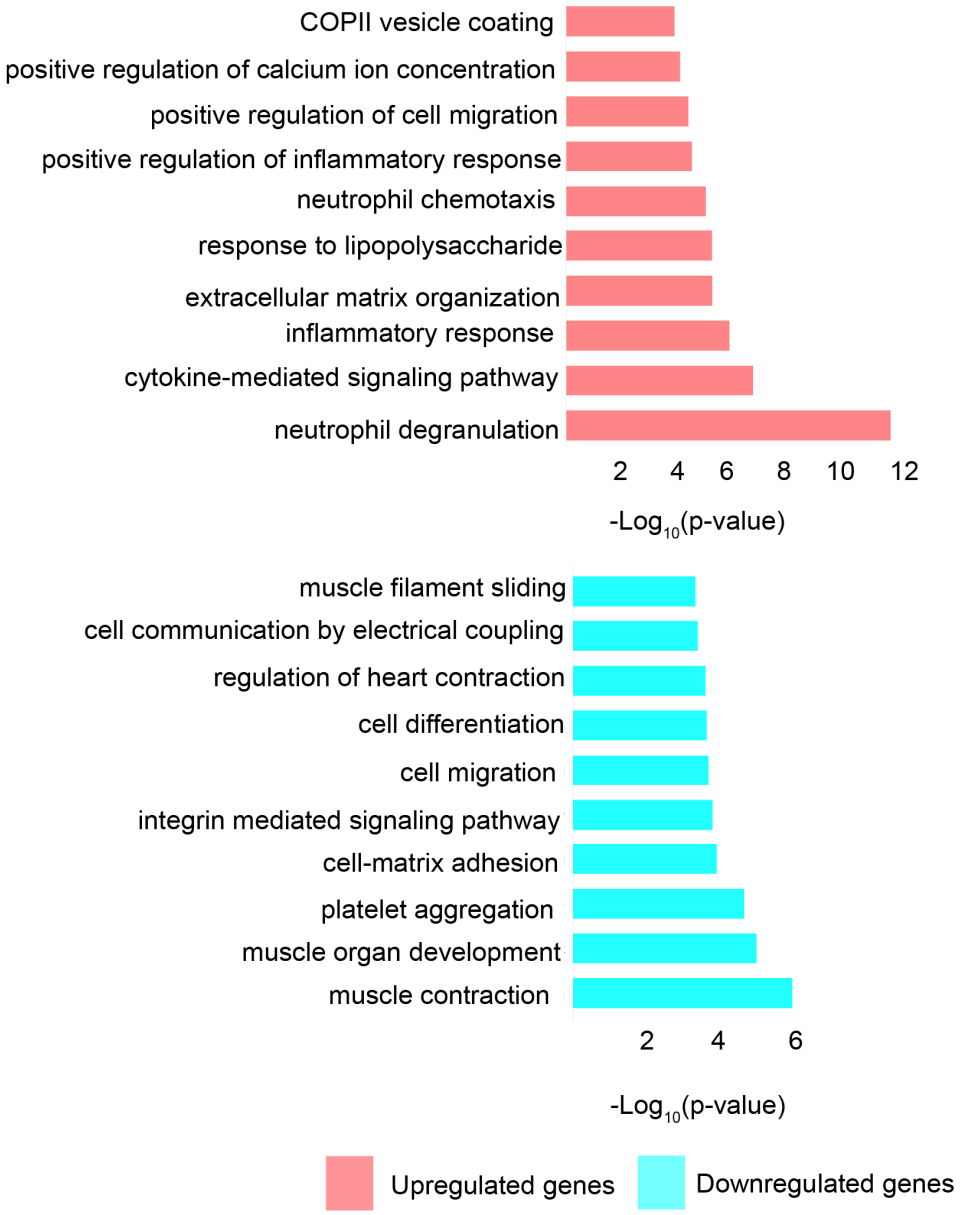


Figure 2-4

Gene ontology of genes upregulated (pink) and downregulated (blue) in labor. Genes upregulated in labor are enriched in calcium signaling and inflammatory response pathways. Genes downregulated in labor are enriched in terms associated with muscle contraction, actin-filament based movement, cell-matrix adhesion, and integrin signaling.

Chapter 3 PROGESTERONE RECEPTOR GENOME-WIDE CHANGES IN PREGNANT MYOMETRIAL TISSUES REVEAL PLAYERS IN LABOR

3.1 Introduction

According to the World Health Organization, preterm birth is a leading cause of infant deaths worldwide. An estimated 15 million babies are born preterm annually worldwide, with approximately 1 million worldwide deaths from PTB complications (Liu et al., 2016). Racial disparities are present in PTB rates, with African American women exhibiting the highest PTB rates compared to non-Hispanic white women, non-Hispanic Asian women, and Hispanic women over the past five years. Despite efforts to prevent PTB, the rate of PTB in the United States continued to steadily increase as recently as 2015 to 2019 (Hamilton et al., 2021). The pathology of PTB is not yet known, and current efforts are only partially successful in prevention. Thus, there is a critical need to understand the pathobiology of PTB to develop preventive or therapeutic strategies for this disease.

The steroid hormone progesterone plays a crucial role in myometrial smooth muscle, such as establishing and maintaining pregnancy. The importance of progesterone in the maintenance of pregnancy is demonstrated by spontaneous pregnancy loss with PGR antagonists at any stage of pregnancy (Avrech et al., 1991). This has been seen with PGR antagonists such as mifepristone (RU486) and PGR modulators, such as ulipristal acetate, the emergency contraception (Glasier et al., 2010). Many mammals show a decline in circulating progesterone levels proceeding labor initiation. This decline in serum progesterone led to the hypothesis that the abatement of progesterone initiates labor. However, circulating progesterone levels remain high in humans during gestation, which led to a functional progesterone withdrawal theory whereby progesterone, acting through its receptor, PGR, is no longer functional in maintaining pregnancy through an unknown mechanism (Boroditsky et al., 1978; Merlino et al., 2007). It was proposed that various PGR isoforms played roles in the control of labor. Recent findings suggest that changes in PGR

isoform B versus A are important for the switch of progesterone signaling from repressive to active on the transcription of labor associated genes (Condon et al., 2008; Nadeem et al., 2016; Nadeem et al., 2017).

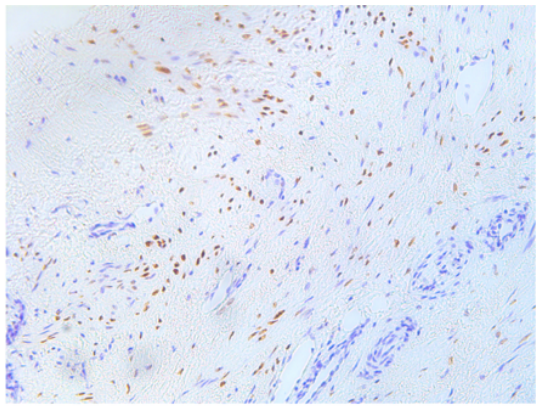
Transcriptomic profiles, found by RNA-seq using a low number of reads, from term pregnant not laboring and laboring myometrial tissues are distinct, and a significant number of differential transcripts have been previously identified (Stanfield et al., 2019). Additionally, distinct transcriptomes of myometrium from pregnant and nonpregnant women are also observed, and the integration of distinct and shared PGR binding sites with differentially expressed genes found between the two groups has provided insight into critical PGR target pathways in pregnancy and, potentially, the labor cascade (Wu et al., 2020). Yet, further information is required to investigate the role of PGR on the transcription of genes necessary for the switch from quiescent myometrium to active myometrium in labor.

In this study, we explored this phenomenon in vivo using myometrial tissues from women who are term in labor (TIL; >37 weeks with signs of labor) and term not in labor (TNIL; >37 weeks with no signs of labor) to determine the progesterone/PGR target genes which are differentially expressed between pregnant myometrial tissues. We performed an *in vivo* assessment of genome-wide PGR binding using the chromatin immunoprecipitation (ChIP)-sequencing (seq) procedure on TIL and TNIL women's tissues. Here, we evaluate whether functional PGR binding in myometrial tissue drives labor by association with genes that play a role in myometrial activation and contraction.

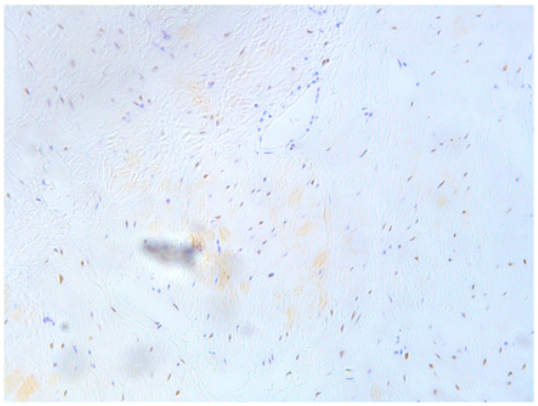
3.2 Results

Differential genome-wide PGR binding between term in labor and not in labor myometrium

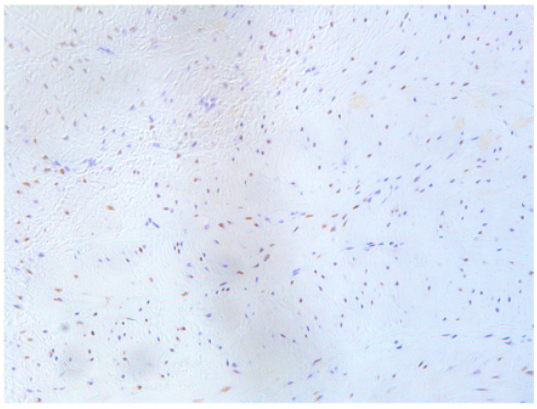
To better understand the molecular mechanisms of the critical roles of the progesterone/PGR pathway in pregnancy and labor, we performed PGR ChIP-seq to profile genome-wide PGR binding in TIL and TNIL myometrial tissues. Prior to that, PGR protein levels were compared between non-pregnant (NP) myometrium and TIL and TNIL myometrium using immunohistochemistry (IHC) (Figure 3-1). Immunostaining of myometrium tissues showed lower cell density in TIL and TNIL myometrium versus NP myometrium, probably due to myometrial cell hypertrophy and increased cell volume during pregnancy. Although the staining intensity for PGR protein seemed substantially higher in NP myometrial tissue, the ratio of PGR positive cells to total cells per high-power field was not different between NP, TIL, and TNIL myometrial tissues (Fig 3-1). On the other hand, the tissue mRNA levels of PGR measured by real-time quantitative polymerase chain reaction (RT-qPCR) were significantly lower in TIL or TNIL compared with NP myometrium (Figure 3-2). Taken together, these data suggest that mRNA and protein levels of PGR in myometrial cells were downregulated in pregnant myometrial tissue, which may be associated with the distinct functions of PGR at different reproductive stages through altering its interaction with chromatin.



NP



TNIL



TIL

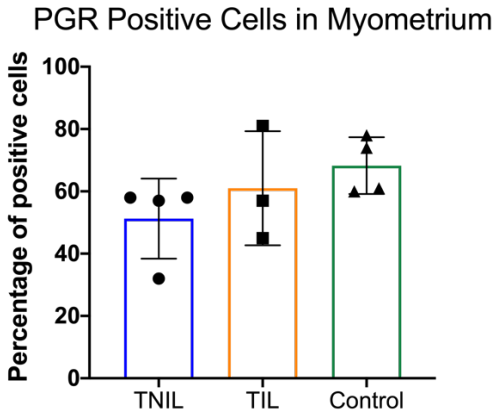


Figure 3-1

Immunohistochemistry of non-pregnant and term pregnant human myometrial tissue biopsies for PGR

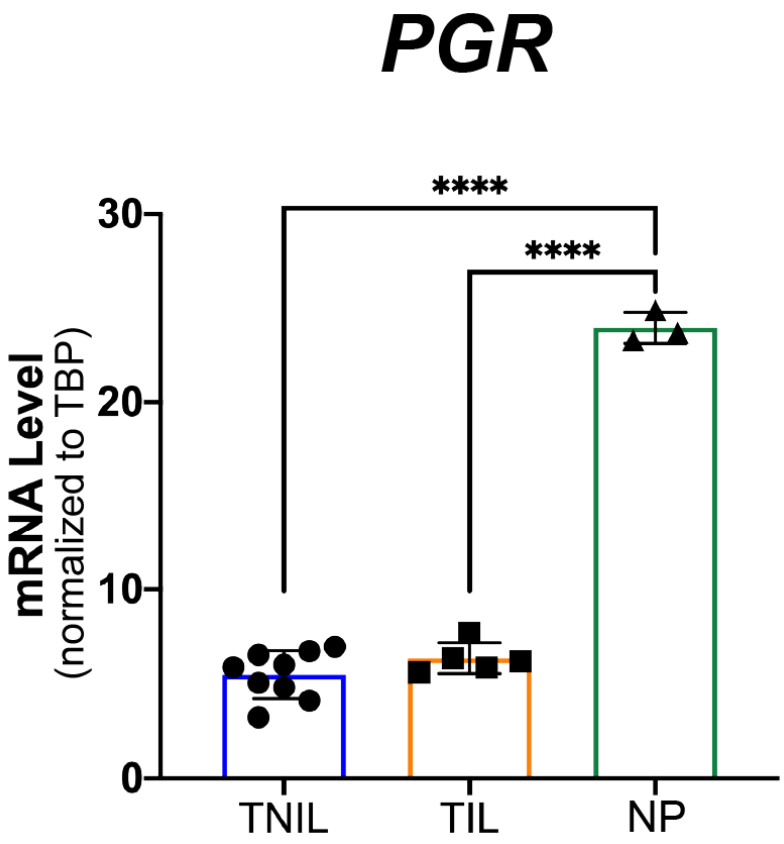


Figure 3-2

mRNA levels of PGR in pregnant term human myometrium and non-pregnant human myometrium

To assess the genome-wide role of myometrial PGR, myometrial tissues from six individual patients (3 TIL and 3 TNIL) were subjected to PGR ChIP-seq. As seen with RNA-seq, clinical characteristics of patients showed no statistical differences between maternal age, parity, BMI, and gestational age among the groups for ChIP-seq (Table 3-1). Consensus PGR peaks in each group were identified and annotated using DiffBind (Ross-Innes et al., 2012) and ChIPseeker (Yu et al., 2015), respectively (Figure 3-3). Over 21,000 peaks were found in TNIL samples and over 34,000 in TIL samples. Peak annotation revealed different genomic distributions of PGR binding sites between TIL and TNIL myometrium. PGR preferentially occupied distal intergenic/enhancer regions compared to other genomic regions in TNIL myometrium but not in TIL myometrium (58.33% vs. 18.9%). In contrast, PGR bound to promoters preferentially vs. distal intergenic/enhancer regions in TIL myometrium (48.53% vs. 15.78%) (Figure 3-4), suggesting PGR binding follows distinct patterns dependent on labor status.

	Maternal age (years)	Parity	BMI (kg/m^2)	GA at delivery (weeks)
PGR ChIPseq				
TNIL (n=3)	34.67 ± 2.33	1 ± 0.58	29.01 ± 3.45	38.53 ± 0.68
TIL (n=3)	37.00 ± 4.16	0.67 ± 0.33	33.02 ± 2.55	38.70 ± 0.40
p-value	0.657	0.649	0.407	0.845
Histone ChIPseq				
TNIL (n=2)	33.33 ± 2.33	2.33 ± 0.33	31.76 ± 4.50	39.00 ± 0.00
TIL (n=2)	39.00 ± 4.00	1.00 ± 0.00	35.68 ± 2.40	39.40 ± 0.10
p-value	0.364	0.057	0.501	0.156

Data are expressed as mean ± SEM. TNIL, term not in labor; TIL, term in labor; BMI, body mass index; GA, gestational age. p-values are from ttests between TNIL and TIL for each -omic experiment.

Table 3-1

Clinical characteristics of myometrial specimens for ChIP-seq

In unanimity with distinct binding patterns, motif analysis uncovered distinct transcription factor binding motifs dependent upon labor status (Figure 3-5). Only 12 motifs were significantly enriched in the TNIL PGR binding sites, whereas 228 motifs were enriched in the TIL PGR binding sites. A classical PGR response element was found only among the TIL peaks, where top enriched motifs included CCCTC Binding Factor (CTCF) and CCCTC Binding Factor Like (BORIS), which play critical roles in chromatin remodeling and epigenetic regulation in the uterine environment (Figure 3-5) (George et al., 2019; Ishihara et al., 2006; Klenova et al., 2002; Loukinov et al., 2002; Rubio et al., 2008; Sun et al., 2008; Wang et al., 2012). Hypermethylated In Cancer Protein (HIC1) is also involved in chromosomal remodeling and can act as a transcriptional regulator (Briggs et al., 2008; van Rechem et al., 2009). Binding sequences for several ETS family members (ELF1, ELK1, ELK4, FLI1) were also enriched as the top motifs around PGR binding sites in TIL myometrium; these factors play significant roles in various biological processes and are modulated by calcium signaling (Figure 3-5) (Comoglio et al., 2003; Dittmer 2003; Liotta et al., 2001; Oikawa and Yamada 2003). Taken together, these findings suggest that PGR may play distinct roles in TIL and TNIL through interaction with distinct transcriptional factors. Myometrial PGR may be associated more loosely with the intergenic regions in TNIL. In labor, however, PGR may bind to promoter regions and classical progesterone response elements more avidly and participate in chromosomal remodeling and epigenetic regulation.

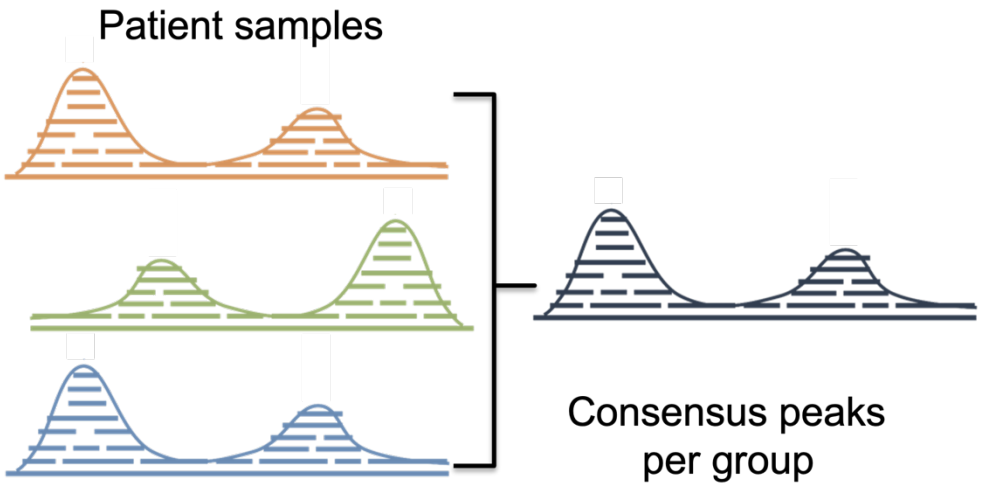


Figure 3-3

Following the identification of peaks for each sample, common or consensus peaks were evaluated due to patient variation. Consensus peaks from the three individual patients per group were found using a method called DiffBind, giving over 21,000 peaks for TNIL and over 34,000 peaks for TIL. These consensus peaks are high confidence peaks that ensure actual PGR target genes for each condition are analyzed for downstream analyses to discern differences between non-laboring myometrium and laboring myometrium.

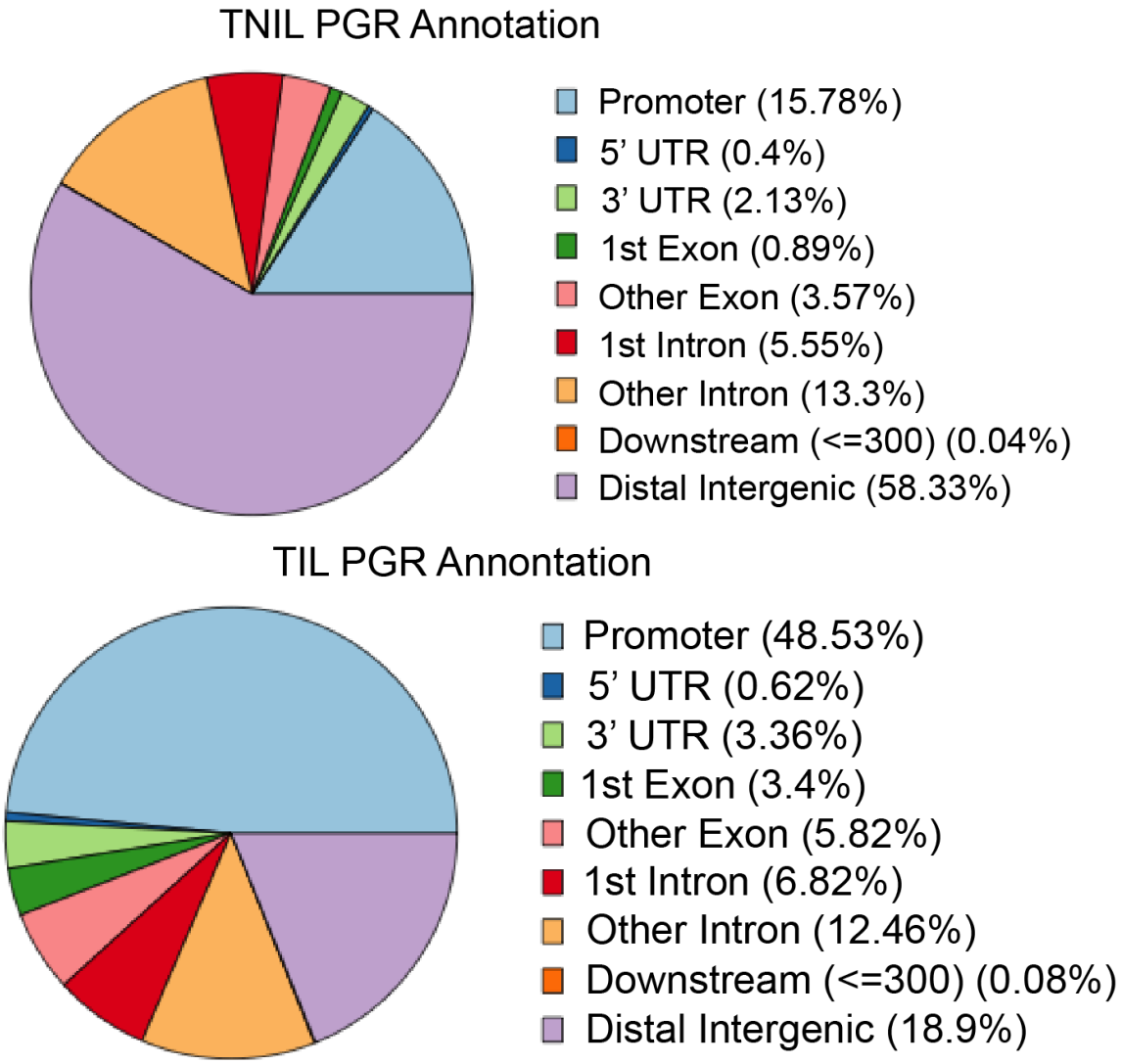


Figure 3-4

Peak annotation for the genomic distributions of PGR binding sites in TNIL (top) and TIL (bottom). PGRs preferentially occupied distal intergenic regions compared to other genomic regions in TNIL myometrium. In contrast, PGR bound to promoters preferentially in TIL myometrium, suggesting that PGR binding follows distinct patterns dependent upon labor status.

MOTIF		Log p-value of enrichment
		TNIL
	ZNF528	-7.91
	HOXA1	-7.14
	VDR	-6.365
	NFAT:AP1	-5.536
	FXR	-5.302
	GATA	-5.278
	Bcl11a	-5.242
	TR4	-5.155
	HIF-1a	-4.989
	Usf2	-4.839
	HIF2a	-4.812
	LEF1	-4.689

Motif Analysis of PGR Binding Sites in TNIL

MOTIF		Log p-value of enrichment
		TIL
	CTCF	-145.8
	BORIS	-87.99
	HIC1	-82.18
	ZNF711	-78.14
	Elk4	-68.23
	ZFX	-66.48
	Elk1	-64.13
	ELF1	-62.31
	Zic3	-60.99
	MYB	-56.84
	Fli1	-52.38
	PGR	-13.26

Motif Analysis of PGR Binding Sites in TIL

Figure 3-5

Motif analysis of TNIL (top) and TIL (bottom) PGR binding sites. Only 12 motifs were discovered in TNIL and over 200 in TIL with a concentration of transcription factors involved in chromosomal remodeling and epigenetic regulation. The progesterone response element was only present in the TIL binding sites.

To assess the labor status more robustly as a determinant for differential genome-wide PGR binding, we again utilized DiffBind. DiffBind analysis uncovered over 1700 differential PGR-bound sites between TIL and TNIL, with 1361 sites gained and 428 lost in labor (Figure 3-6). Functional enrichment analysis found pathways involved in cAMP-mediated signaling enriched in labor (Figure 3-7). On the other hand, focal adhesion, integrin activation, cell-matrix adhesion, and cytoskeleton transport were enriched for genes that lost PGR binding in labor, similar to the pathways found in downregulated genes in labor (Figure 2-4). This suggests the importance of PGR in maintaining extracellular matrix structure in the TNIL group (lost binding in labor, Figure 3-7).

Integration of transcriptome and PGR cistrome uncovers novel target genes in pregnant myometrium

To understand the role of PGR in the regulation of transcripts associated with labor status, we integrated the genes differentially expressed between TIL and TNIL with statistical significance (Figure 2-3) with the PGR binding sites lost in TIL myometrium (Figure 3-6) using CistromeGO ((Li et al., 2019), which uncovered 94 DEGs that were enriched in biological pathways associated with GTPase activity, cell-matrix adhesion, Rap protein signaling, and potassium ion cellular response (Figure 3-8). In contrast, integration of DEGs with gained PGR binding sites in TIL yielded 270 genes potentially regulated by enriched PGR binding in labor (Figure 3-9). These genes were involved in MAPK cascade, histone acetylation, response to pain, negative regulation of hypoxia-induced apoptotic signaling, and cortical actin cytoskeleton organization (Figure 3-9).

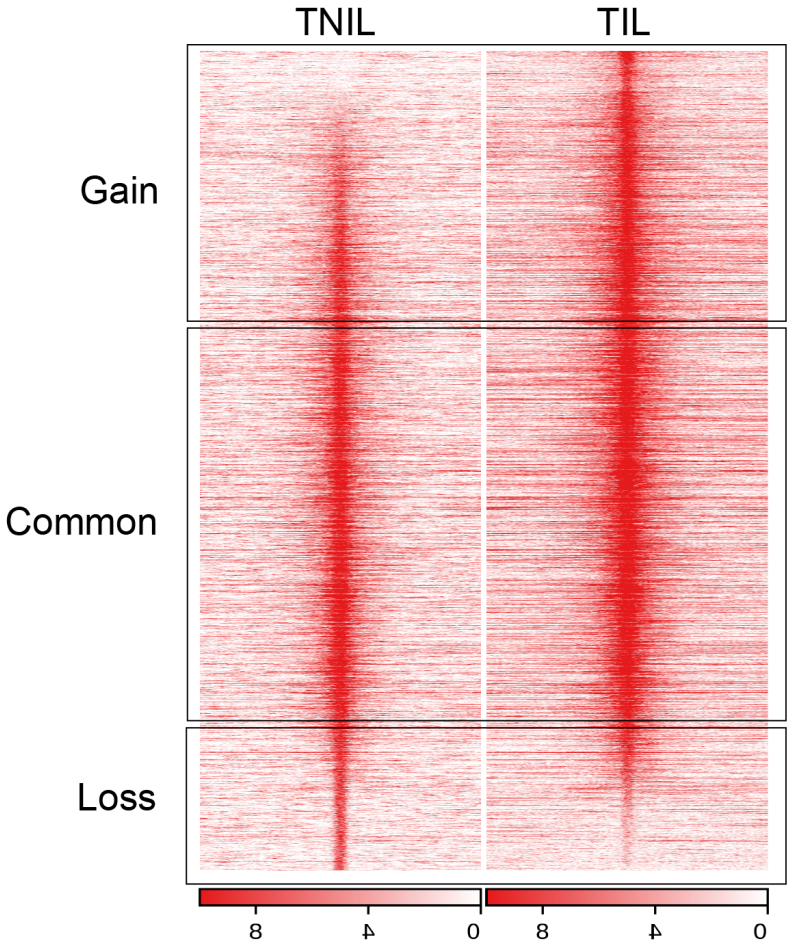


Figure 3-6

Heatmap of 1789 differential peaks between TNIL and TIL, found by DiffBind. Genome-wide PGR binding for TNIL and TIL are plotted, left and right, respectively. Differences in intensity or strength of PGR binding are depicted by the intensity of the color red, as indicated by the scale. Four hundred twenty-eight sites were “lost” in labor, and 1361 sites were “gained.”

Figure 3-7

Functional enrichment of genes containing a gained (pink) or lost (blue) PGR binding site in labor. Genes found to have gained binding in labor were enriched for pathways associated with cAMP-mediated signaling, cell membrane repolarization, negative regulation of CREB activity, and negative regulation of interleukin-2 production. In contrast, genes with loss of PGR binding in labor were enriched in focal adhesion, integrin activation, and cell adhesion pathways.

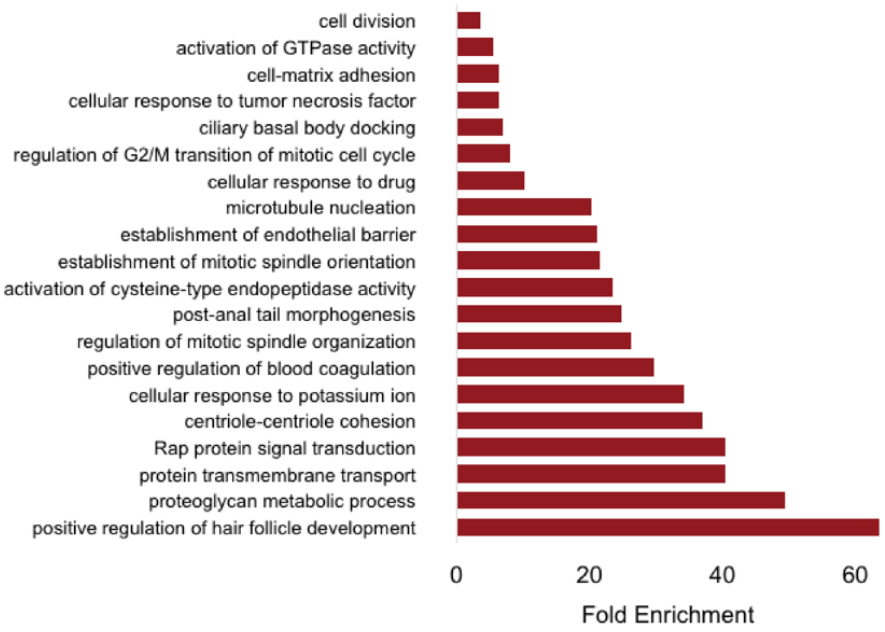


Figure 3-8

Integration of the DEGs found in RNAseq dataset with the PGR binding sites in TNIL, using CistromeGO, uncovered 94 DEGs enriched in biological pathways associated with GTPase activity and potassium ion cellular response. Both are generally critical pathways for muscle relaxation and contraction.

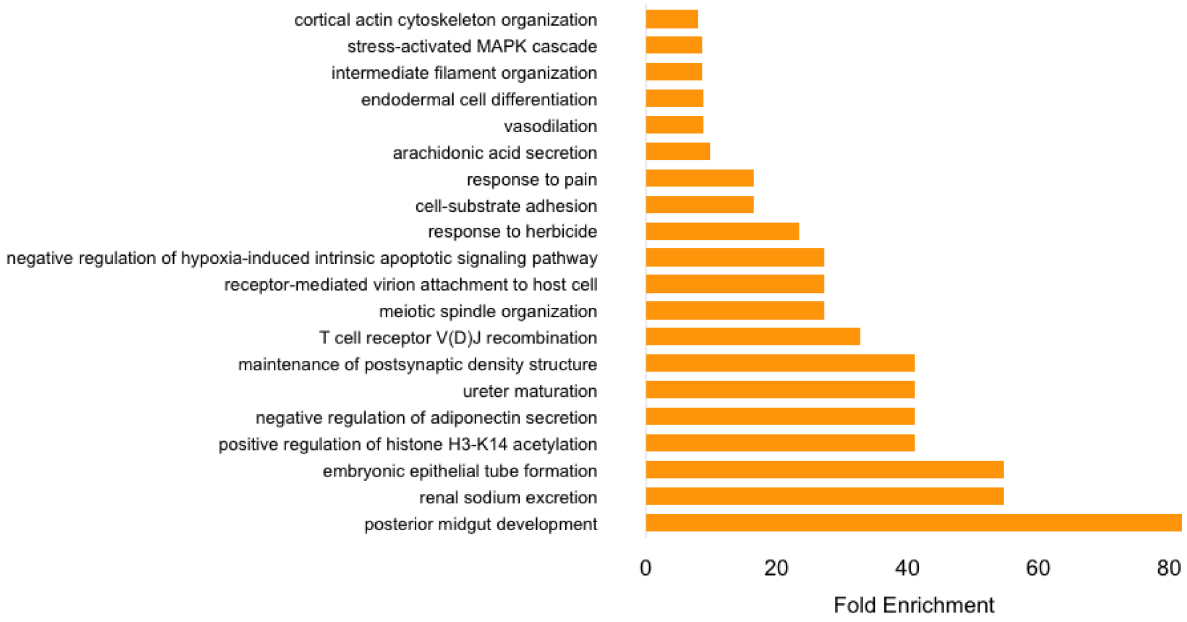


Figure 3-9

Integration of the DEGs found in the RNAseq dataset with the PGR binding sites in TIL, using CistromeGO, uncovered 270 DEGs that were enriched in involved in MAPK cascade, histone acetylation, response to pain, negative regulation of hypoxia-induced apoptotic signaling, and cortical actin cytoskeleton organization. Thus, various biological processes in epigenetic regulation, cellular structural support, and cellular organization.

Histone modification binding maps in term pregnant myometrium

The histone modifications H3K4me3 and H3K27ac are associated with transcriptionally active chromatin. We performed H3K4me3 and H3K27ac ChIP-seq in TIL (n=2) and TNIL (n=3) myometrial tissues, separate from the samples used for PGR ChIP. Using average plots, no major differences were detected in H3K27ac binding along gene bodies (Figure 3-10). However, individual samples for H3K4me3 binding did show decreased enrichment of binding in TIL compared to TNIL samples at transcription start sites but other areas of the gene body seem unaffected by labor status (Figure 3-11).

We found 15,012 and 14,640 consensus H3K4me3 peaks in TNIL and TIL, respectively. For H3K27ac, 21,856 and 35,441 consensus peaks were found in the TNIL and TIL groups, respectively. Genome-wide enrichment of the consensus histone mark peaks shows no major difference between labor statuses (Figure 3-12). A previous study in mice showed that active histone marks are already bound to promoter and enhancer regions of labor-associated genes (Shchuka et al., 2020). Thus, we explored the consensus histone mark signatures at the promoters and distal regions of labor-associated genes: *FOS*, *GJA1*, *OXTR*, and *ZEB1*. Consensus peaks for H3K27ac were found in promoter or enhancer regions of these labor-associated genes (*FOS* pictured; Figure 3-13). Furthermore, H3K4me3 was also found in these labor-associated genes within promoter regions (*FOS* pictured; Figure 3-13). This data supports previous findings from mouse non-laboring uteri that labor-associated genes are activated before labor onset.

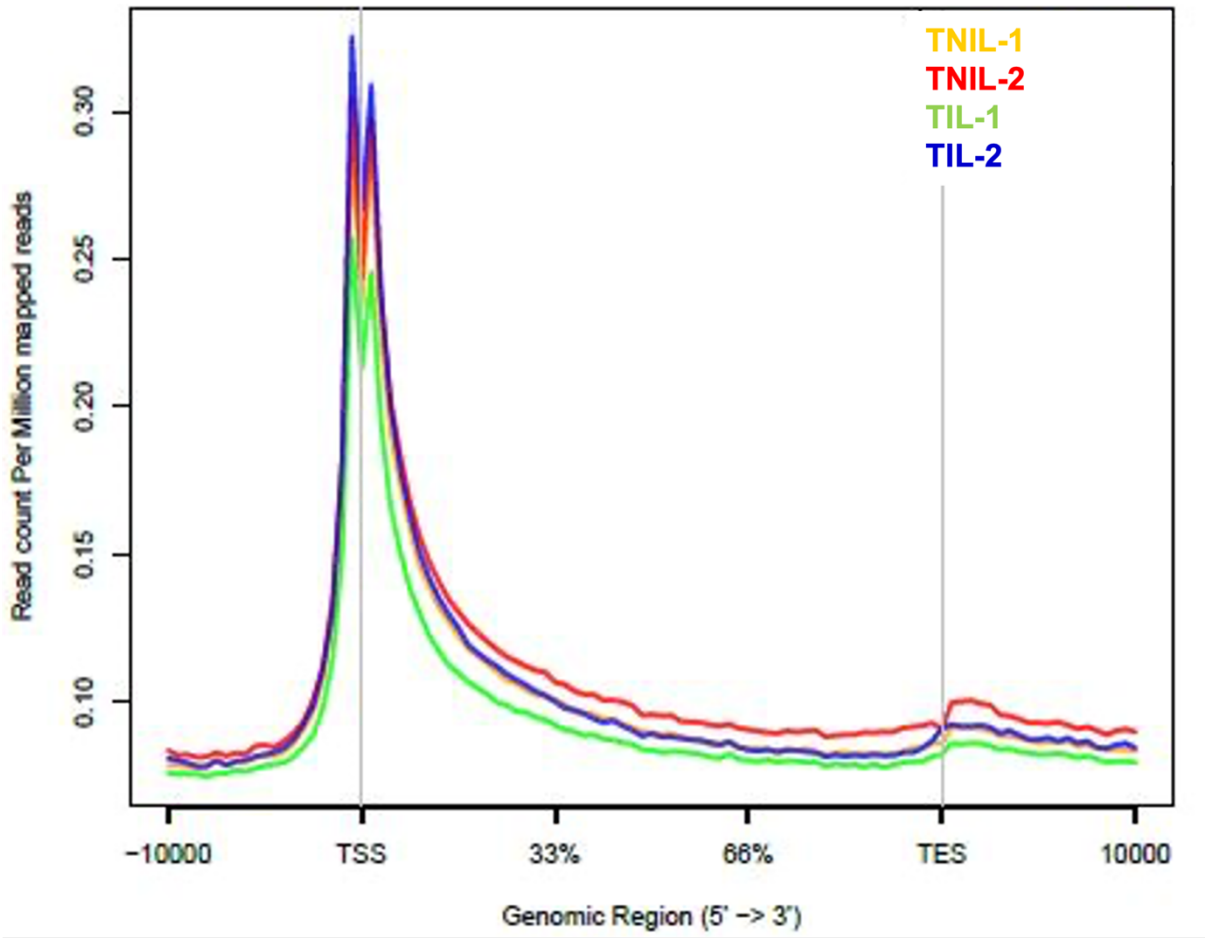


Figure 3-10

Average plot depicting histone modification H3K27ac binding in two TNIL and two TIL samples

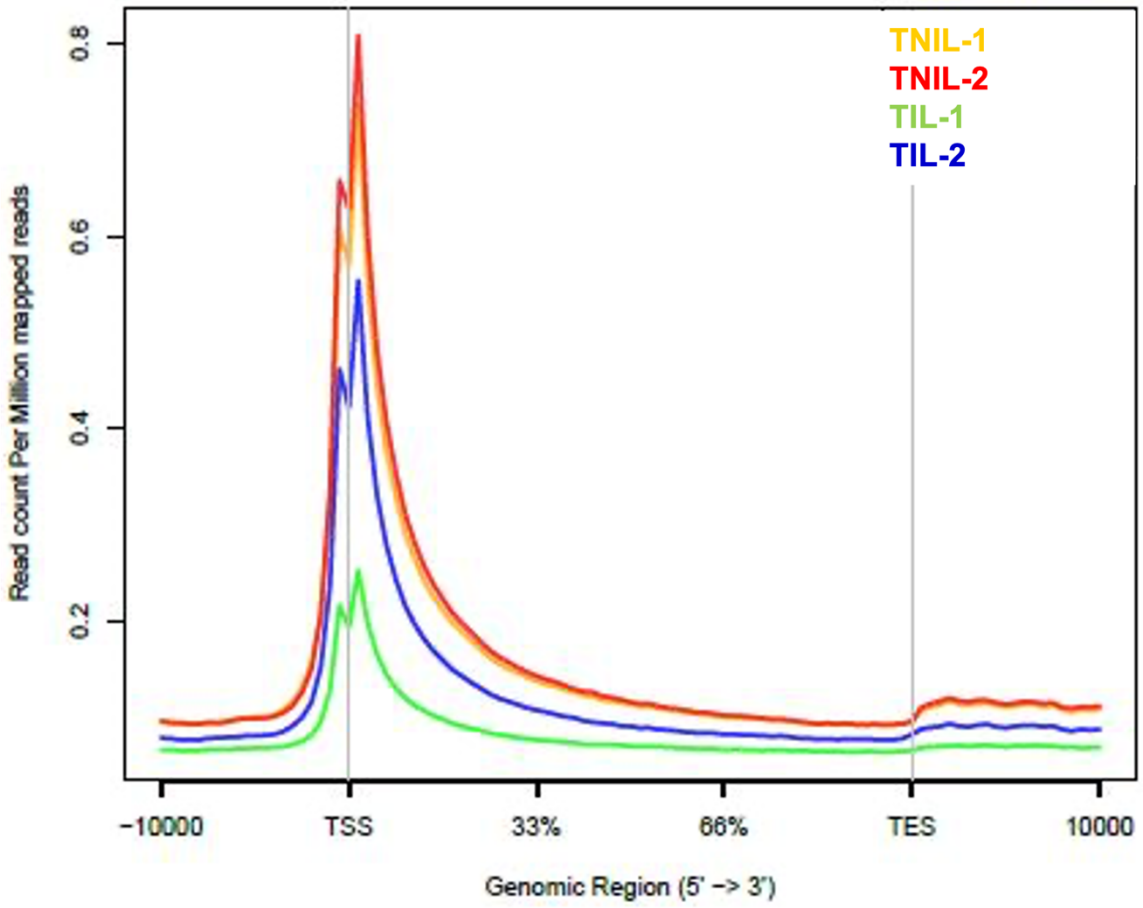


Figure 3-11

Average plot depicting histone modification H3K4me3 binding in two TNIL and two TIL samples

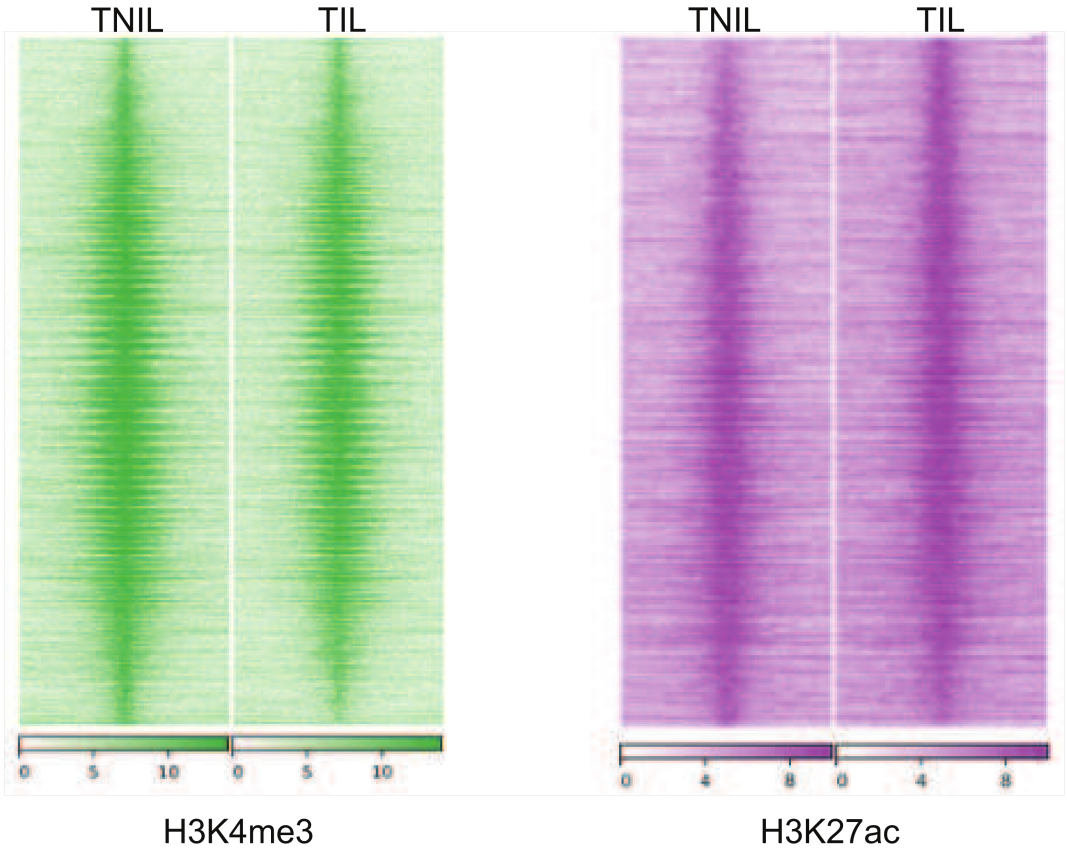


Figure 3-12

Heatmap of genome-wide H3K4me3 and H3K27a binding in TNIL and TIL myometrium

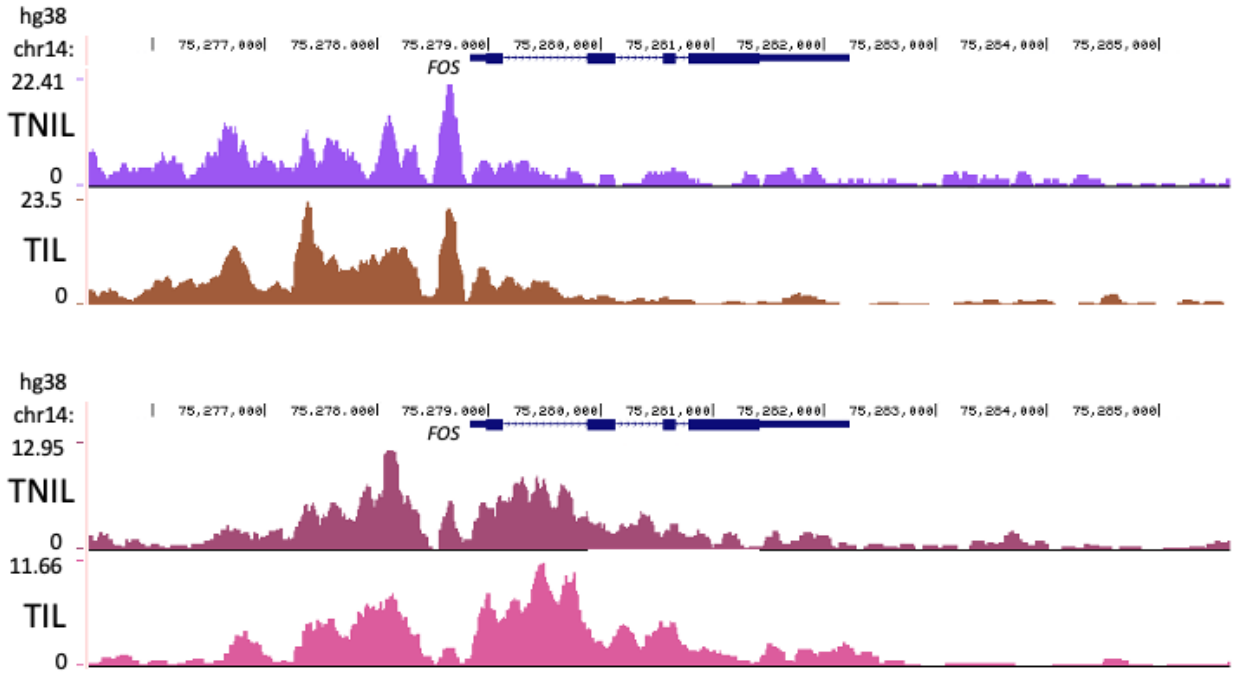


Figure 3-13

H3K27ac (top) and H3K4me3 (bottom) consensus reads at the labor-associated gene *FOS* in TIL (brown; pink) and TNIL (purple; magenta)

Because H3K27ac indicates active transcription at promoters and enhancers, enrichment of transcription factor binding motifs was performed in TIL and TNIL consensus peaks using HOMER. Promoter regions were further examined for H3K4me3 in TIL and TNIL myometrium. HOMER identified 168, 161, 240, and 241 enriched motifs in H3K27ac distal regions and H3K4me3 promoter regions in TIL and TNIL, respectively (Table 3-1, 3-2). Among these motifs, 22 and 15 were uniquely enriched in the H3K27ac distal regions of TIL and TNIL when comparing labor status (Table 3-1) and 49 and 50 were uniquely enriched in the promoter regions of H3K4me3 binding in TIL and TNIL, respectively (Table 3-2). Most of the transcription factor motifs found for both H3K27ac distal regions and H3K4me3 promoter regions encoded ETS proteins, basic leucine zipper domain (bZIP) proteins, and zinc fingers. Their functions in pregnant myometrium have yet to be elucidated. A portion of transcription factor motifs from TIL PGR peaks (Figure 3-5) overlapped with the H3K27ac and H3K4me3 binding motifs in TIL. Furthermore, the PRE was found in H3K27ac distal binding motifs in both TIL and TNIL myometrium. Therefore, we examined the overlap of PGR binding in pregnant myometrium with H3K27ac distal regions and H3K4me3 promoter regions. Of the 34,938 PGR peaks in labor, only 1.9% of these peaks overlapped with an H3K27ac enhancer region (Table 3-3). However, 35.2% of PGR labor peaks overlap with H3K4me3 regions within promoters (Table 3-4).

Group	Histone Mark	Number of Enriched Motifs	List of Uniquely Enriched Motifs
TIL	H3K27ac	168	AP2-gamma, Bcl11a, bHLHE40, BORIS, CLOCK, EHF, ERG, FOXK1, Gfi1b, HLF, Hoxc9, MyoD, NFAT, p63, Rfx6, RUNX-AML, RUNX, STAT4, STAT6, Tcf21, TEAD3, TR4
TNIL	H3K27ac	161	E2F, EBNA1, HIF-1a, HIF2a, MITF, Nr5a2, p53, PAX5, SF1, Six4, Sp1, Srebp1a, Srebp2, ZFX

Table 3-1

Enriched motifs found in TIL and TNIL myometrium from H3K27ac peaks in distal regions

Group	Histone Mark	Number of Enriched Motifs	List of Uniquely Enriched Motifs
TIL	H3K4me3	240	AMYB, Bapx1, Bcl6, BMYB, CRX, CTCF-SatelliteElement, EHF, Eomes, Fox:Ebox, Foxo1, Hand2, HNF4a, Hoxa11, Hoxa13, Hoxa9, Hoxd11, HRE, Isl1, MafB, Meis1, MYB, Nkx3.1, Nkx6.1, Pitx1, RUNx-AML, RUNX, RUNX1, RUNX2, SCRT1, Sox10, Sox15, Sox17, Sox2, Sox3, Sox4, Sox6, Sox9, STAT4, Tbet, Tbr1, Tbx21, Tbx6, TEAD1, TEAD3, TEAD4, Tgif1, Tgif2, ZNF189, ZNF41
TNIL	H3K4me3	241	Arnt:Ahr, bHLHE40, bHLHE41, BORIS, c-Myc, CRE, CTCF(Zf), DUX, E-box, E2F, E2F1, E2F3, E2F4, E2F6, E2F7, Egr1, Egr2, ELF1, Elk1, ETS, ETV1, ETV4, Fli1, HIF-1a, HIF-1b, HIF2a, HINFP, KLF10, KLF14, KLF3, Klf4, KLF5, KLF6, Klf9, LRF, Maz, NRF, NRF1, Sp1, Sp2, Sp5, Tcfcp2l1, Usf2, WT1, ZBTB12, ZBTB33, Zfp281, ZKSCAN1, ZNF264, ZNF519

Table 3-2

Enriched motifs in TIL and TNIL myometrium from H3K4me3 peaks in promoter regions

	H3K27ac Enhancer Region Peaks	Overlapping PGR Enhancer Peaks	% of PGR Peaks
TNIL	19,996	101	0.5%
TIL	17,567	661	1.9%

Table 3-3

Overlapping H3K27ac and PGR peaks within enhancers

	H3K4me3 Promoter Region Peaks	Overlapping PGR Promoter Peaks	% of PGR Peaks
TNIL	49,913	1,369	6.2%
TIL	31,567	12,298	35.2%

Table 3-4

Overlapping H3K4me3 and PGR peaks within promoters

Using DiffBind, a portion of consensus peaks were found as differentially bound by the active histone marks in myometrium. We identified 1138 and 2082 genes associated with differential H3K4me3 and H3K27ac enrichment, respectively, in these two tissues (Figure 3-14; Figure 3-15). Gene ontology revealed pathways related to relaxation of vascular smooth muscle, negative regulation of cAMP-dependent protein kinase activity, and negative regulation of focal adhesion assembly to be enriched amongst genes with differential binding of H3K27ac. Moreover, inflammatory response, interleukin-2 mediated signaling, regulation of cardiac muscle contraction by calcium ion signaling, and cell communication by electrical coupling were among some of the pathways enriched from genes found with differential H3K4me3 binding. This suggests that the specificity of histone binding in pregnant myometrium may have specific functions related to pathways involved in immune response and smooth muscle contraction (Somlyo and Somlyo 2003; Wu et al., 2008).

Three-way integration analysis reveals potential target genes

To pinpoint functional PGR target genes, we performed a 3-way integration analysis. The DEGs with differential PGR binding were integrated with the differential histone modification sites, revealing 17 candidate genes (Table 3-5; Table 3-6). We focused on three genes involved in calcium signaling and actin/myosin pathways because they have an established importance in smooth muscle contraction. The gene that encodes ATPase phospholipid transporting 11A

(ATP11A; Figure 3-16) was found to be highly expressed in TIL with enriched PGR binding and H3K27ac modification compared with TNIL, suggesting that PGR binding may stimulate its expression in labor (Tsuchiya et al., 2018). In contrast, CBX7 and TNS1 (Figure 3-17; Figure 3-18). were downregulated with enhanced PGR binding but decreased active histone (H3K4me3 and H3K27ac) modification sites in TIL, suggesting that PGR binding may inhibit their expression (Bernau et al., 2017; Yap et al., 2010). We also noted that KIF5C, a member of the kinesin superfamily of molecular motors and a regulator of local translation, showed increased mRNA levels, PGR binding, and promoter/enhancer histone modifications (Table 3-5; Table 3-6). Although the physiologic roles of KIF5C in uterine smooth muscle cells are not known, its expression was reported to be upregulated in uterine leiomyomas (Swarnkar et al., 2021).

Differential H3K27ac	Differential PGR	Differential Expression	Gene Symbol
Up	Up	Up	COLEC12, ATP11A, NID2, CHST15, KIF5C
Up	Down	Up	TMED10
Down	Up	Down	MPRIP, NCS1, TNS1, ALDH4A1
Down	Down	Down	SVIL, RERG, MAP4, EVA1C, GRIN2A

Up, upregulated or enriched in TIL
Down, downregulated or not enriched in TIL

Table 3-5

Summary of genes from integration of differential gene expression, PGR occupancy, and H3K27ac occupancy between TIL and TNIL

Differential H3K4me3	Differential PGR	Differential Expression	Gene Symbol
Up	Up	Up	KIF5C
Up	Down	Up	None
Down	Up	Down	TNS1, CBX7, AHDC1
Down	Down	Down	None

Up, upregulated or enriched in TIL
Down, downregulated or not enriched in TIL

Table 3-6

Summary of genes from integration of differential gene expression, PGR occupancy, and H3K4me3 occupancy between TIL and TNIL

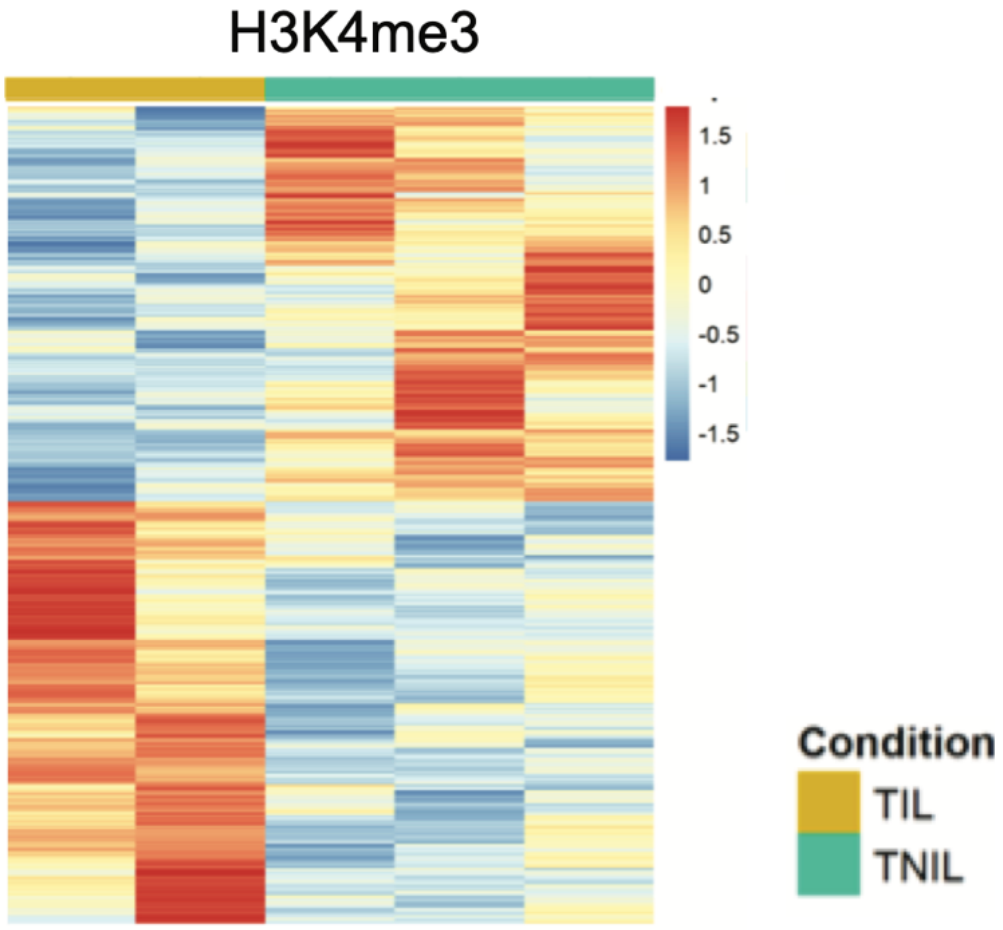


Figure 3-14

Heatmap depicting differential binding analysis for H3K4me3 shows that 550 genes gained histone binding in labor and 588 lost histone binding in labor. The colors represent the degree of gained or lost binding, in red and blue, respectively.

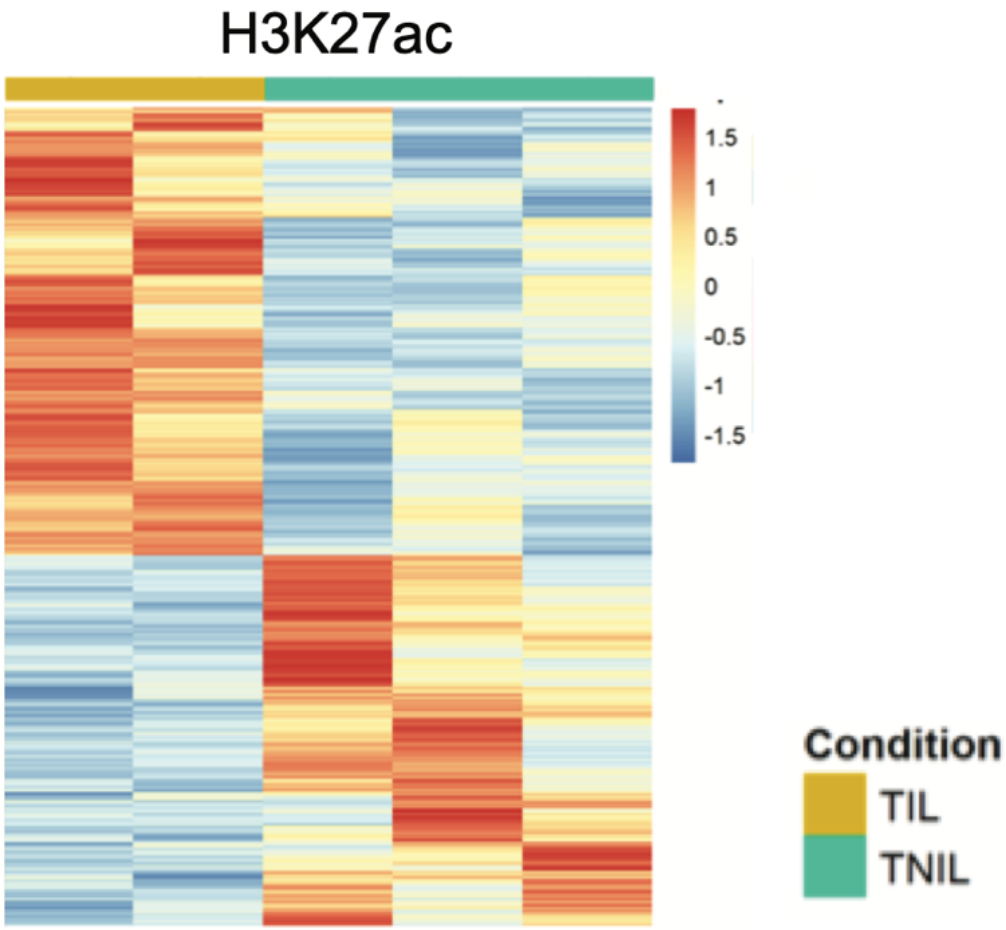


Figure 3-15

Heatmap depicting differential binding analysis for H3K27ac shows that 1140 genes gained histone binding in labor and 942 lost histone binding in labor. The colors represent the degree of gained or lost binding, in red and blue, respectively.

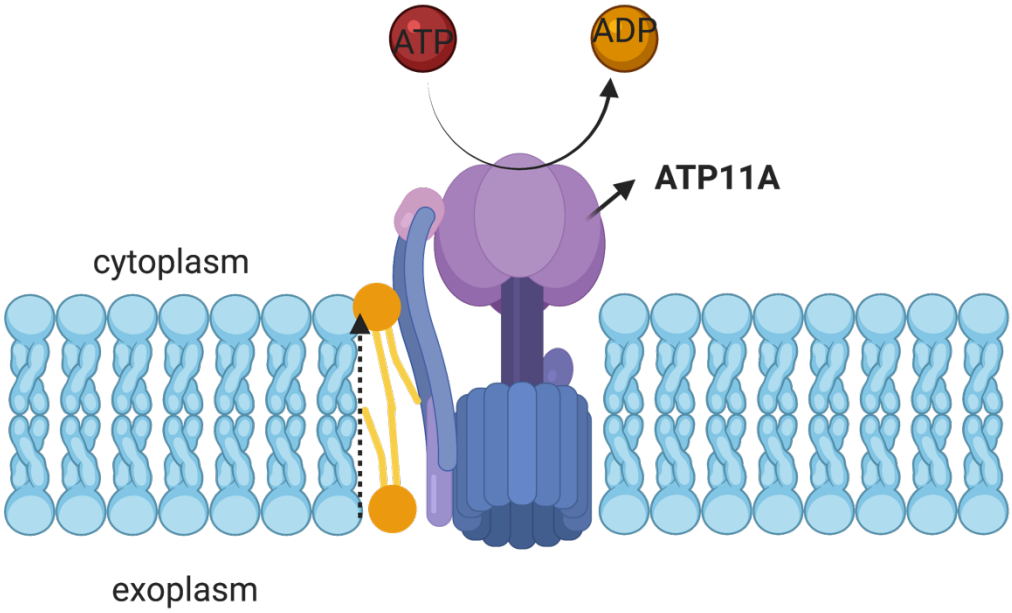


Figure 3-16

ATP11A is an ATPase that catalyzes the hydrolysis of ATP coupled to transporting phosphatidylserines. This phospholipid transporter protein stimulates calcium influx and Rho GTPase signaling, leading to smooth muscle contraction, assembly of myosin fibers, and myotube formation in myoblasts.

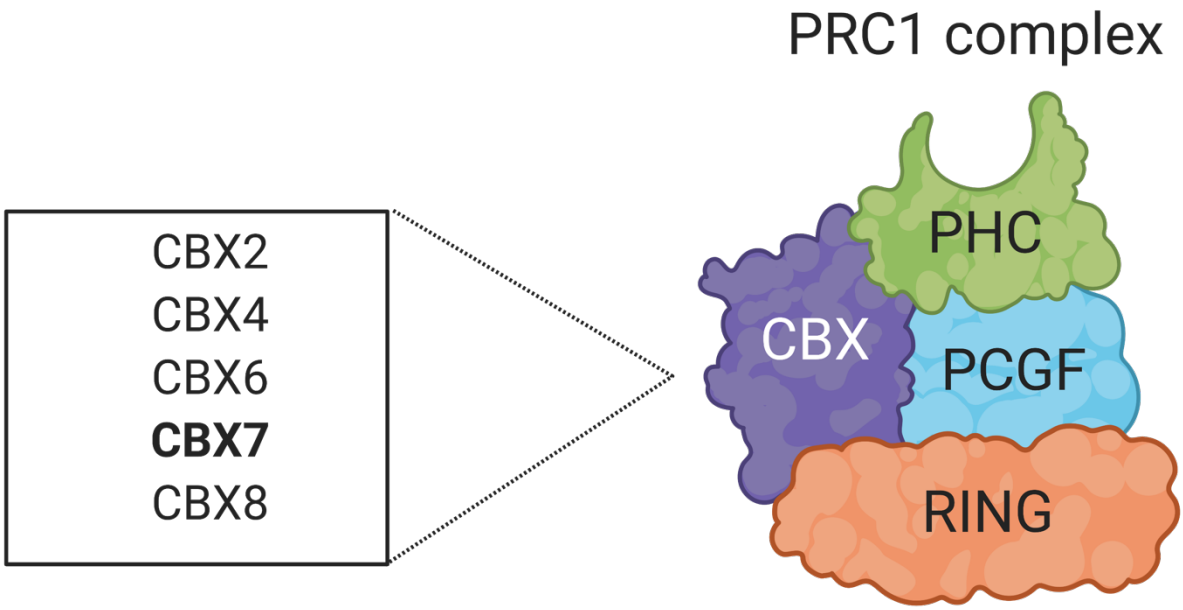


Figure 3-17

CBX7 is the gene that encodes the Chromobox 7 protein, a canonical component of the polycomb group in the PRC1 complex critical for transcriptional repression of many genes

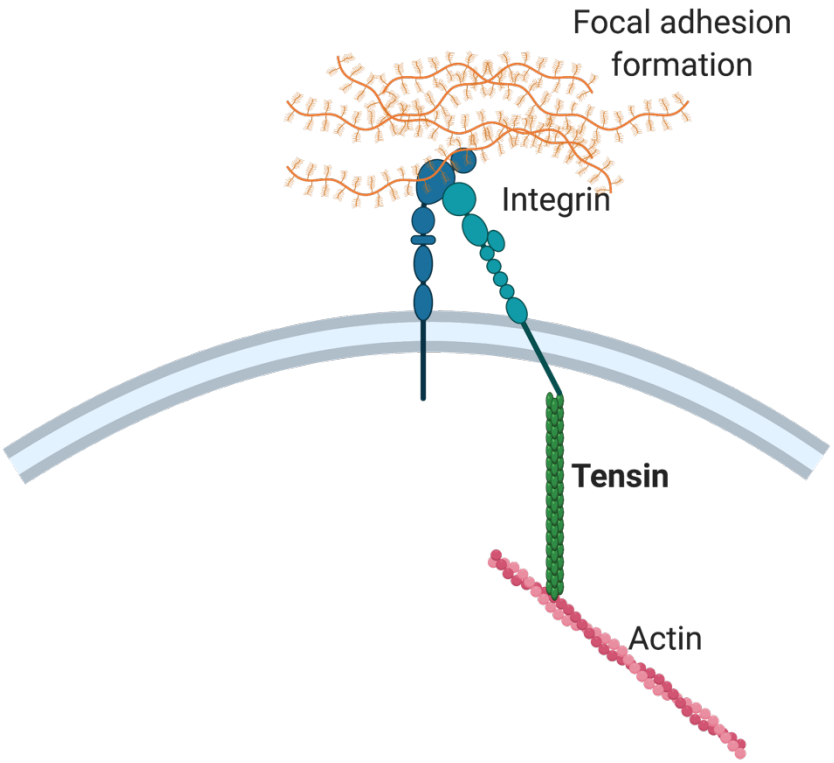


Figure 3-18

Tensin-1 (TNS1) is a focal adhesion protein that is a substrate of Calpain II, which cleaves cytoplasmic and nuclear substrates in the presence of intracellular Ca2+ overload. It is also involved in tethering actin to integrin and forming focal adhesions required for actin-based traction.

Regulation of PGR labor associated genes in human myometrial cells

We assessed the regulation of expression of three candidate genes by progesterone using immortalized pregnant human myometrial cells (PHM1-41). The cells were treated with progesterone (P4, 10^{-5} M) for 24 hours (n=3). P4 treatment significantly upregulated the mRNA levels of ATP11A and CBX7 and downregulated that of TNS1 expression without any significant effects on PGR mRNA expression (Figure 3-19). The results suggest that ATP11A, CBX7, and TNS1 are P4/PGR target genes. The validation studies may best be performed using *in vivo* experimental systems, which remains outside the scope of this study and should be considered in future directions.

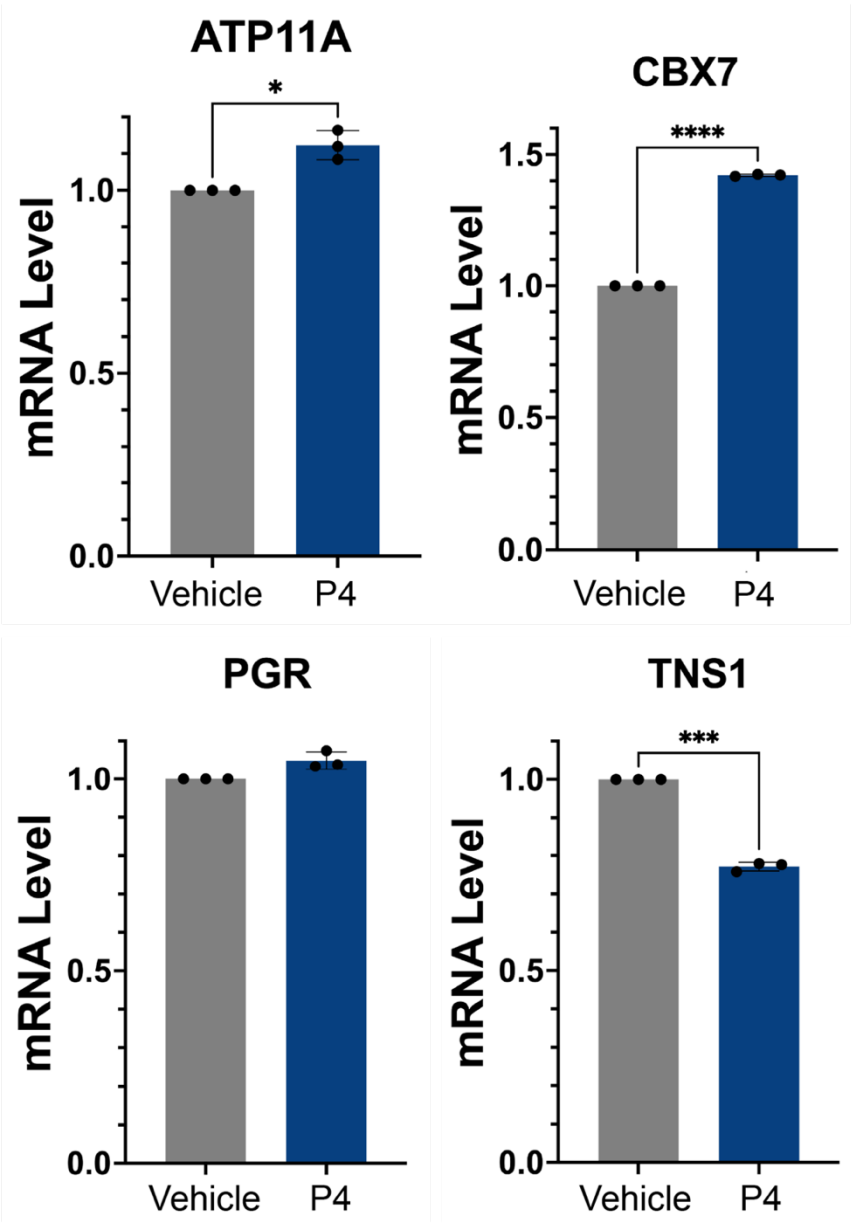


Figure 3-19

qPCR results measuring the mRNA levels of candidate genes in immortalized pregnant human myometrial cells (PHM1-41) treated with progesterone (P4, 10^{-5} M) or vehicle for 24 hours (n=3). P4 treatment significantly upregulated the mRNA levels of ATP11A and CBX7 (top) and downregulated that of TNS1 expression (bottom left), without any significant effects on PGR mRNA expression (bottom right).

Chapter 4 UNWRAPPING ESR1 BINDING MAPS IN PREGNANT MYOMETRIAL TISSUES TO REVEAL POTENTIAL CONTRIBUTORS IN PARTURITION

4.1 Introduction

The steroid hormone estradiol is also thought to play an essential role in pregnancy and labor. During pregnancy, estradiol levels are drastically increased compared to before pregnancy. Estriol levels consistently rise 2-4 weeks before the onset of labor, suggesting the importance of estrogen in labor (Boroditsky et al., 1978). Estrogen receptor alpha (ERα) is increased in laboring myometrium, suggesting that ERα activation promotes contractility and excitability of the myometrium. COX2 is a gene that encodes an enzyme important for producing prostaglandins, which trigger the release of oxytocin from the pituitary gland. Increases in oxytocin levels do not coincide with the onset of labor; however, increases in the receptor for oxytocin (OXTR) and density of OXTR have been observed in the myometrium of pregnant women at the onset of labor (Kimura et al., 1996). COX2 and OXTR are categorized as CAPs responsible for the myometrial activation required for labor. These CAPs are induced by estrogen and increase dramatically prior to the onset of labor in human myometrium (Mesiano et al., 2002). Increases in *COX2* and *OXTR* mRNA correlate with increased ERα expression, suggesting that these CAPs are target genes (Mesiano et al., 2002).

In the previous chapter, PGR binding dynamics in pregnant myometrium were explored and distinguished by labor status. In this study, the binding dynamics of ESR1 in vivo using myometrial tissues from women who are term in labor (TIL; >37 weeks with signs of labor) and term not in labor (TNIL; >37 weeks with no signs of labor) to determine the estrogen/ESR1 target genes which are differentially expressed between pregnant myometrial tissues are analyzed.

4.2 Results

Differential genome-wide ESR1 binding between term in labor and not in labor myometrium

Before genome-wide ESR1 binding identification, the samples from PGR IHC (Figure 3-1) were also subjected to ESR1 IHC (Figure 4-1). Immunostaining of myometrium tissues showed significantly reduced density in TIL and TNIL myometrium compared to NP myometrium, while no difference was observed between term labor status. Quantification of the ratio of positive cells versus total cells resulted in statistically significant differences only between NP myometrium and pregnant myometrium (Figure 4-2). However, RT-qPCR measurement of mRNA levels shows that ESR1 mRNA is significantly different between all three groups (Figure 4-3). ESR1 was significantly higher in NP myometrium than term pregnant myometrium, and overall, TIL had higher expression than TNIL myometrium, consistent with previous findings (Mesiano et al., 2002; Welsh et al., 2012; Mesiano and Welsh, 2007). The differences between the pregnant myometrium may be associated with the activation of CAPs in the laboring myometrium.

To assess the genome-wide role of myometrial ESR1, tissues from the same six individual patients in which PGR ChIP-seq was performed were also subjected to ESR1 ChIP-seq. Statistical analysis of the patient characteristics showed no statistical differences in maternal age, parity, gestational age, and BMI among the two groups (Table 3-1). Consensus ESR1 peaks for each group were identified and annotated as previously described (Chapter 3.2, Figure 3-3). In TIL, 28,690 peaks were found as high confidence peaks, while 35,420 peaks were found in TNIL. Peak annotation revealed similar genomic distributions among ESR1 binding sites. Thus labor status did not influence ESR1 genomic binding patterns (Figure 4-4).

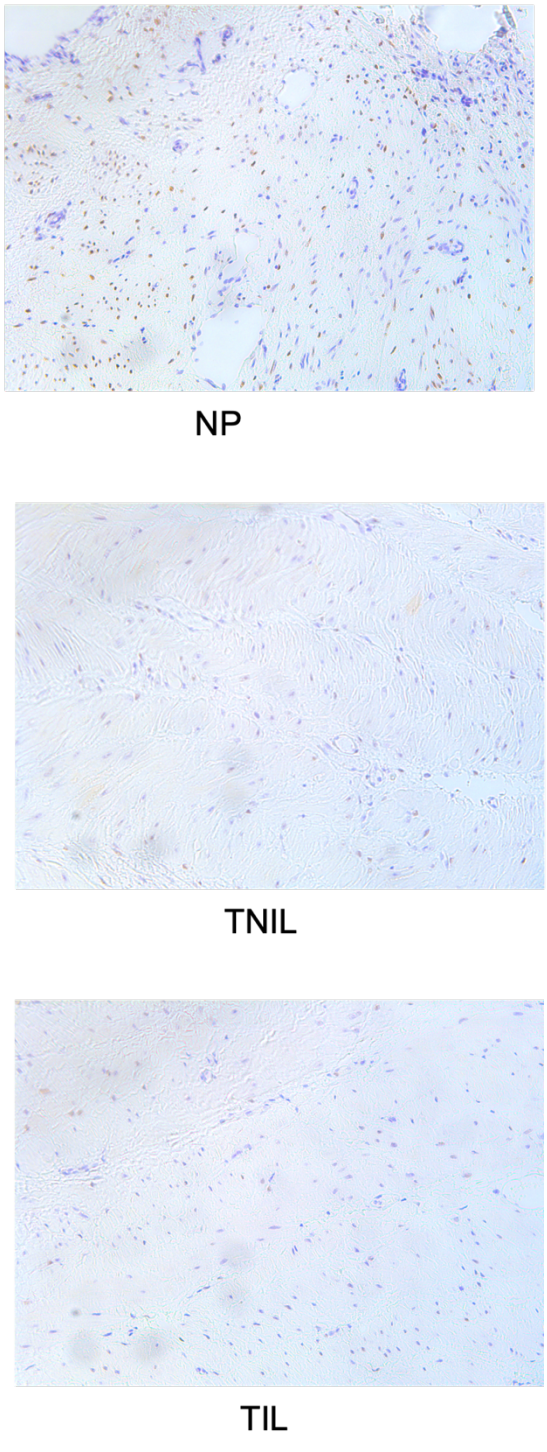


Figure 4-1

IHC of ESR1 in non-pregnant and pregnant myometrium.

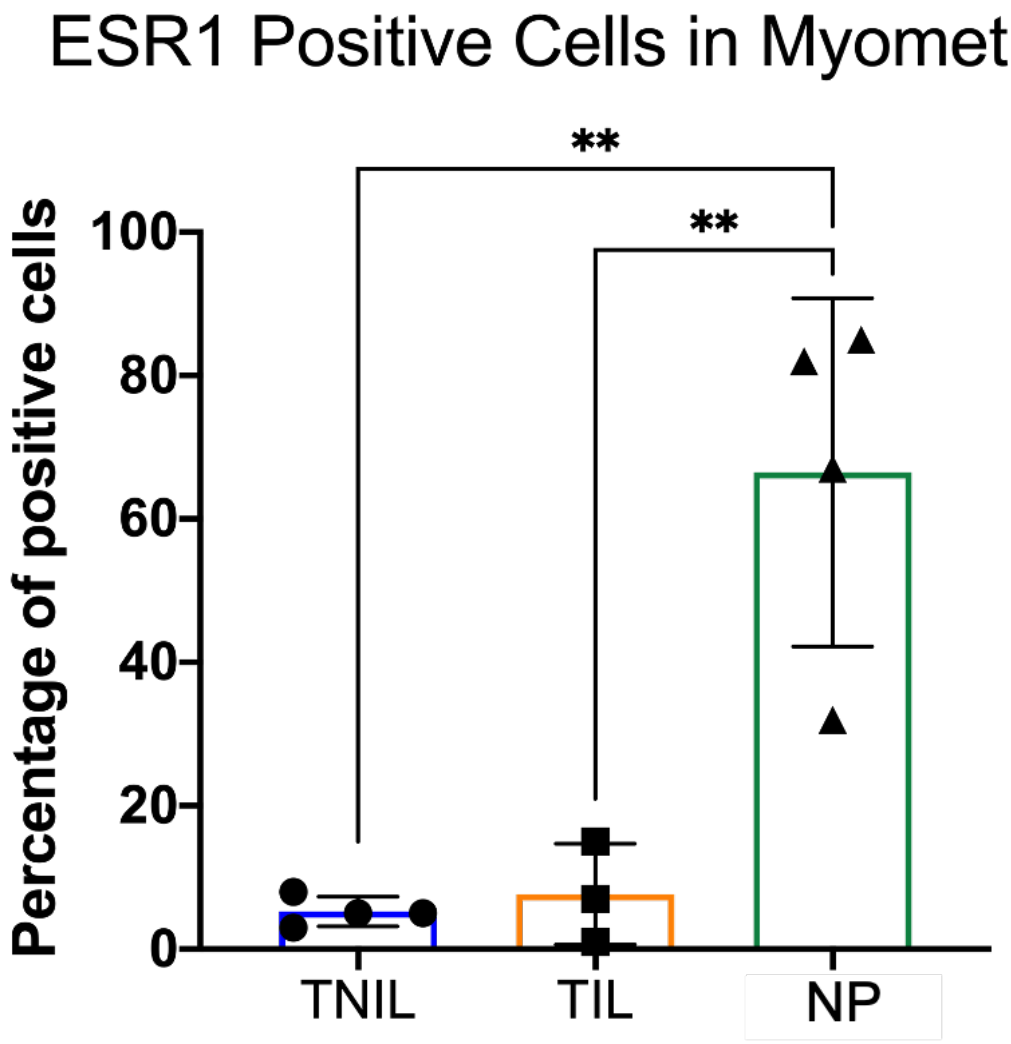


Figure 4-2

Quantification of ESR1 positive cells from IHC.

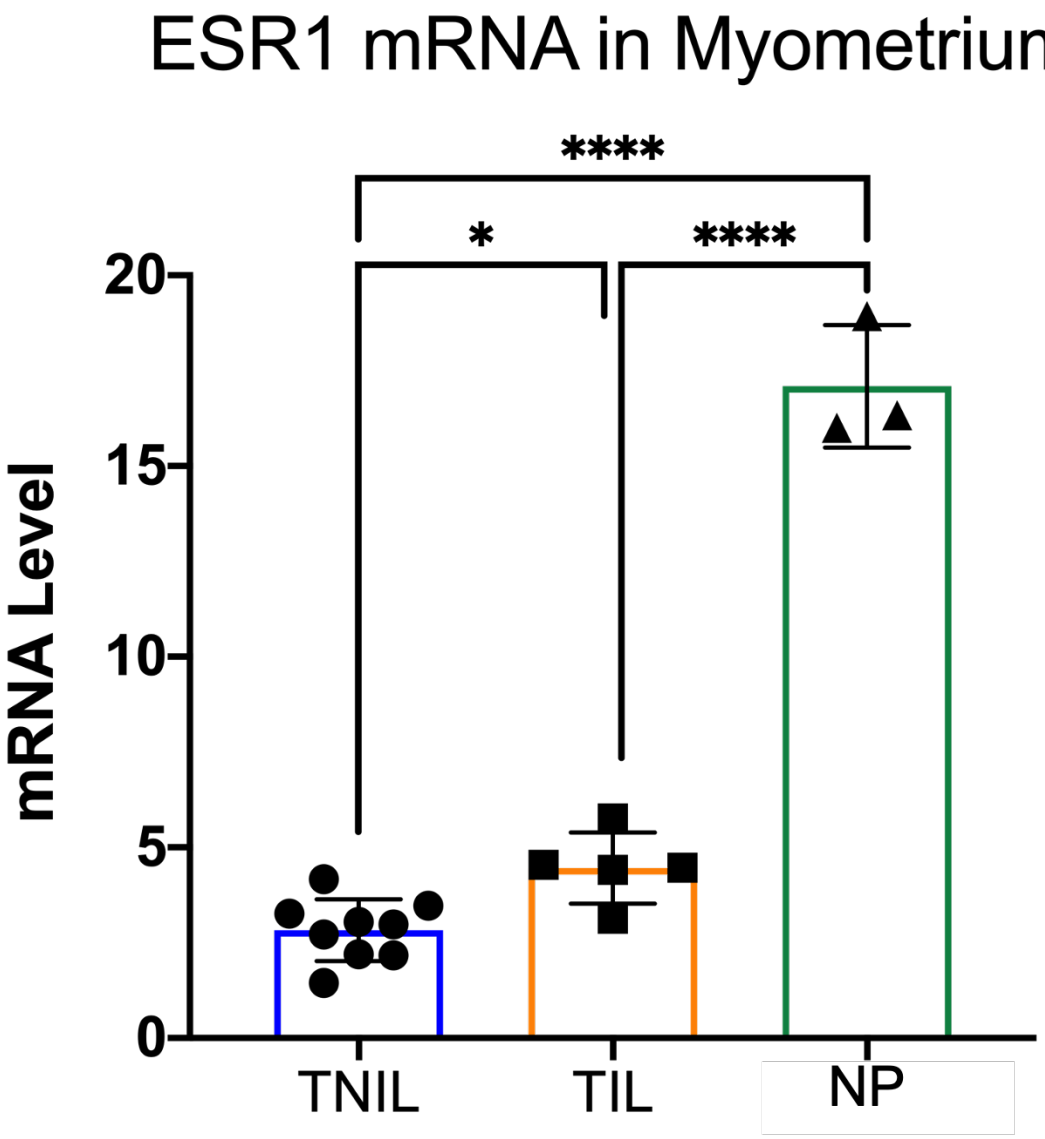


Figure 4-3

mRNA levels of ESR1 in TIL, TNIL, and NP myometrium.

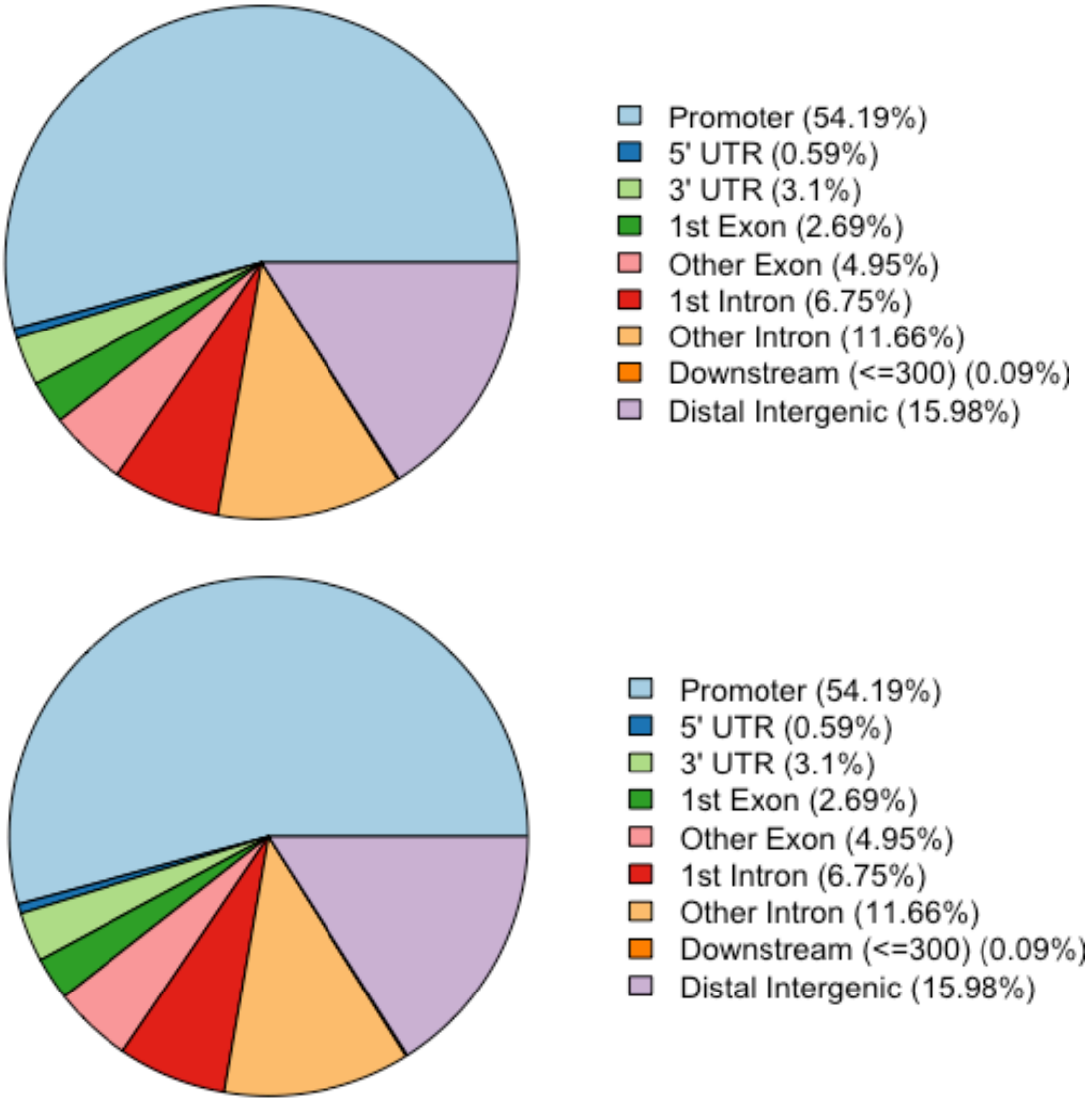


Figure 4-4

Peak annotation for the genomic distributions of ESR1 binding sites in TNIL (top) and TIL (bottom). ESR1 preferentially occupied promoter regions in TIL and TNIL myometrium, suggesting that ESR1 binding does not follow distinct patterns dependent upon labor status.

Motif analysis provided similar results. ESR1 bound preferentially to ETS factors and chromatin remodelers to a higher degree of significance than PGR TIL results mentioned in the previous chapter, regardless of labor status (Figure 4-5). The estrogen response element (ERE) was found in both groups with comparative statistical significance. Taken together, these results suggest that labor status does not influence ESR1 chromatin-binding dynamics.

Despite these similarities, DiffBind was again utilized to discern differentially ESR1 bound genes by labor status. This analysis uncovered 1499 genes, with 655 genes gained in labor and 844 lost in labor (Figure 4-6). Gene ontology of differentially bound sites revealed genes found to have gained binding in labor were enriched for neuronal associated pathways and the establishment of chromosome localization. In contrast, genes with a loss of ESR1 binding in labor were enriched in CDC42 signaling. CDC42 was necessary for human extravillous trophoblast invasion, a process critical for the normal establishment of the placenta (Nicola et al., 2008). Additionally, calcium signaling and stress-activated protein kinase signaling pathways, the latter implicated in cytokine regulation and apoptosis in human placenta, were also enriched in ESR1 lost sites in labor (Cindrova-Davies et al., 2007; Figure 4-7).

MOTIF		Log p-value of enrichment
		TNIL
	CTCF	-258.7
	BORIS	-185.6
	ZNF711	-83.8
	ZFX	-82.1
	Elk1	-80.3
	HIC1	-78.3
	Elk4	-73.9
	ELF1	-71.2
	Zic3	-62.4
	ERE	-15.6

Motif Analysis on TNIL ESR1 Peaks

MOTIF		Log p-value of enrichment
		TIL
	CTCF	-344.9
	BORIS	-217
	Elk4	-120
	Fli1	-106.4
	Elk1	-100.3
	ELF1	-99.4
	ETV4	-95.7
	c-Myc	-89.2
	n-Myc	-87
	ERE	-15.5

Motif Analysis on TIL ESR1 Peaks

Figure 4-5

Motif analysis of TNIL (top) and TIL (bottom) ESR1 binding sites. 205 motifs were discovered in TNIL and 215 in TIL with a concentration of transcription factors involved in chromosomal remodeling and epigenetic regulation as the topmost enriched motifs, same as the PGR TIL motif analysis results. The estrogen response element was present in both groups and the same level of significance.

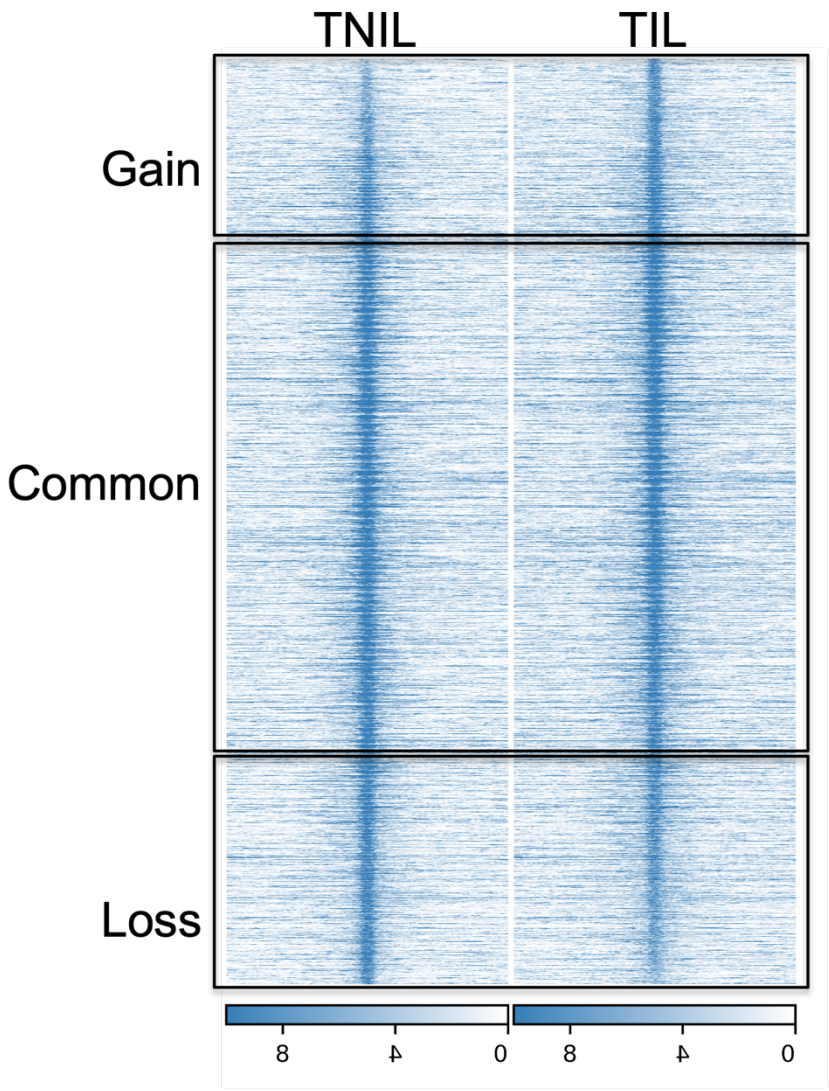


Figure 4-6

Genome-wide ESR1 binding in TNIL (left) and TIL (right). It is important to note that statistical analysis was used to differentiate common/consensus and differential peaks between TNIL and TIL. This visual representation may not reflect the degree of common and different as best as possible

Figure 4-7

Functional enrichment of genes found to contain a gained (pink) or lost (blue) ESR1 binding site in labor. Genes found to have gained binding in labor were enriched for pathways associated with establishment of chromosome localization. In contrast, genes with loss of ESR1 binding in

labor were enriched in CDC42, calcium signaling, and stress-activated protein kinase signaling pathways

Integration of transcriptome and ESR1 cistrome uncovers novel target genes in pregnant myometrium

To further grasp the role of ESR1 in the differentiation of the labor phenotype, integration of DEGs (Figure 2-3) with ESR1 differential binding (Figure 4-6) using CistromeGO was performed to find potential ESR1 regulated genes. This analysis uncovered a total of 154 genes that were differentially expressed between TIL and TNIL and contained an ESR1 binding site lost in labor (Figure 4-8). These genes were involved in lipopolysaccharide mediated signaling, leukocyte migration, and phosphorylation pathways. Interestingly, I-kappaB-, an anti-inflammatory enzyme, associated pathway was also found. The role of estrogen and pro- or anti-inflammatory responses in the myometrium has not yet been addressed to have any insinuations in laboring human myometrium yet a link between the two in other models has been explained as multi-factorial and complex (Straub et al., 2007; Tian et al., 2017). A comparable number of genes, 160, were found in the integration of DEGs with gained ESR1 binding sites in TIL (Figure 4-9). In contrast, these genes were enriched in biological pathways associated with muscle contraction, cytoskeleton organization, cell adhesion, angiogenesis, and damaged tissue response. These results suggest that estrogen may be associated to CAPs despite muscle contraction already occurring is this group. Moreover, with the dramatic tissue changes associated with labor, estrogen is responding to tissue damage in TIL where the need is the most due to forceful contractions associated with labor. Further integration with histone ChIP-seq (Figure 3-10; Figure 3-11) resulted in a total of 15 candidate genes (Table 4-1).

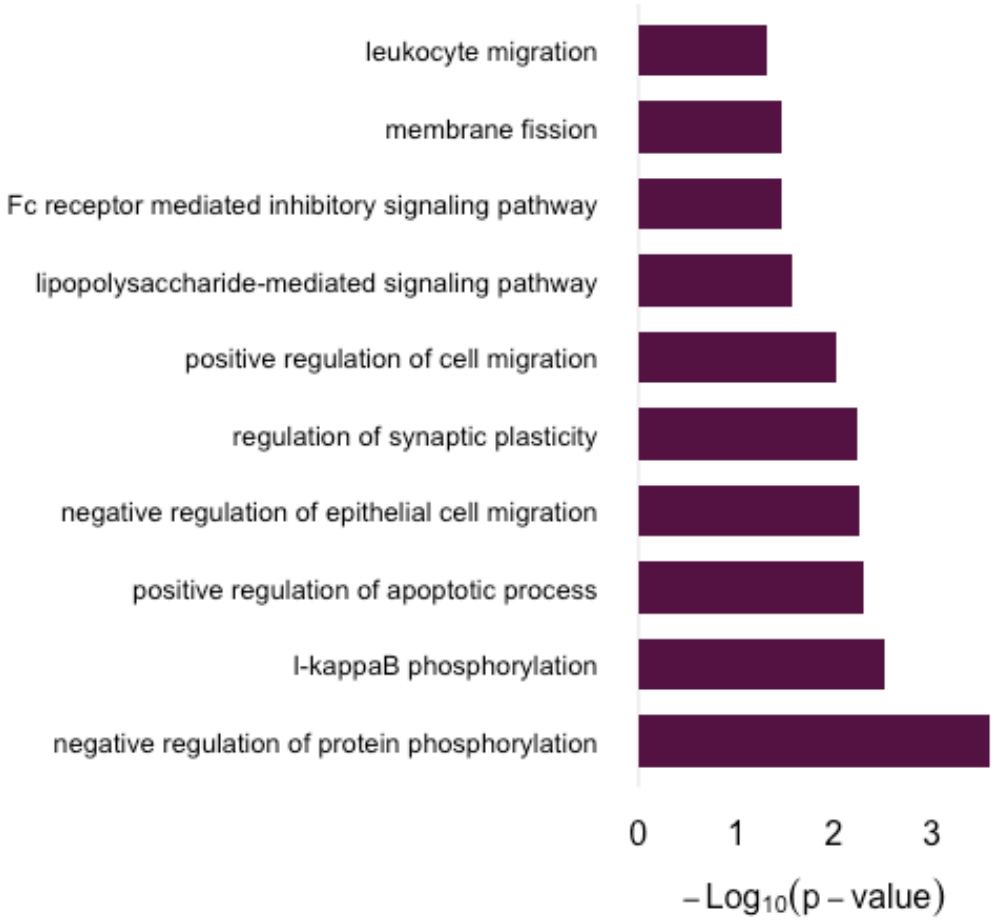


Figure 4-8

Integration of the DEGs found in RNAseq dataset with the ESR1 binding sites in TNIL, using CistromeGO, uncovered 154 DEGs that were enriched in biological pathways associated with inflammatory and phosphorylation pathways.

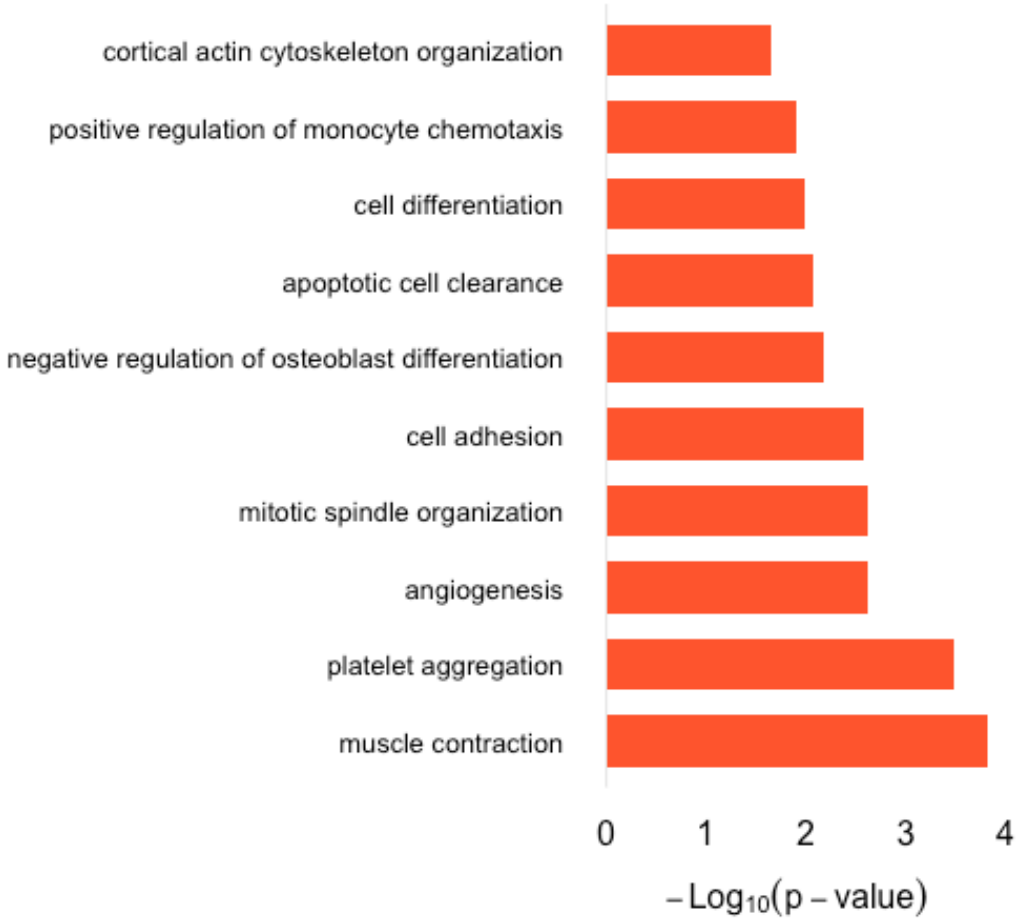


Figure 4-9

Integration of the DEGs found in RNAseq dataset with the ESR1 binding sites in TIL, using CistromeGO, uncovered 160 DEGs that were enriched in biological pathways associated with muscle contraction, cytoskeleton organization, cell adhesion, and damaged tissue response.

Differential H3K27ac	Differential ESR1	Differential Expression	Gene Symbol
Up	Up	Up	PTCH1, ERI1, CACNA1D
Up	Down	Up	None
Down	Up	Down	SVIL, SCG2, MAP4, EVA1C, TTC28, VCL
Down	Down	Down	TRIO, ERC1, ALDH4A1, PRUNE2, GRIN2A, RAB40A

Up, upregulated or enriched in TIL

Down, downregulated or not enriched in TIL

Table 4-1

List of candidate genes for further validation studies

Chapter 5 MATERIALS AND METHODS

Subject Criteria

Women undergoing cesarean section (C-section) at Prentice Women's Hospital of Northwestern Memorial Hospital were consented as per a protocol approved by the Institutional Review Board of Northwestern University. Women were categorized under two groups: term not in labor (TNIL; >37 weeks displaying no signs of cervical dilation or contraction of the uterus), or term in labor (TIL; >37 weeks). Women in the TNIL group had a scheduled elected C-section. Both groups excluded women undergoing C-sections for fetal or maternal stress, breech presentation, or any infection. After informed consent was obtained from each patient, samples were de-identified once tissue was collected.

Tissue Collection

Myometrial tissue (2 x 0.5 x 0.5 cm) was excised from the upper rim of the transverse C-section incision made in the lower uterine segment. The muscle tissue was dissected off the serosa (peritoneum) or endometrium. Then it was immersed in 15 mL of ice-cold PBS in a 50 mL Falcon Tube and washed three times with 15 mL of PBS. After washing tissue, samples were snap-frozen using liquid nitrogen and stored at -80°C before proceeding with RNA isolation and chromatin isolation, while a small portion of tissue was fixed for immunohistochemistry (IHC).

Immunohistochemistry

A small portion of tissue was fixed in Davidson's Fixative overnight and then immersed in fresh 100% ethanol until IHC. Fixed tissues were stained for progesterone receptor (M3569, Agilent)), estrogen receptor α (GA084, Agilent), and IgG as a control.

RNA Isolation

Frozen tissues were homogenized using a mortar and pestle cooled with liquid nitrogen and approximately, 0.35 g to 0.5 g of tissue was used. RNA isolation was performed according to

the mini-RNeasy kit (74104, Qiagen) instructions. RNA concentration and yield were quantified using Nanodrop.

RNA Sequencing (RNA-seq)

DNA library for each RNA sample was constructed using the KAPA RNA Hyper Prep Kit in conjunction with the KAPA Single-Indexed Adapter Kit (KAPA Biosystems). For library amplification 500 ng of RNA was used with an adapter concentration according to KAPA protocol. Libraries were sequenced using 75 base pair single-end reads on the Illumina NextSeq 500 at an average target depth of 33 million reads per sample.

RNA-seq Data Analysis

RNA-seq reads were aligned using STAR aligner (v2.6.3) with default settings (Dobin et al., 2013) to GR38 human genome assembly. Reads count per gene was performed using htseq (Anders et al., 2015). Differential gene expression was detected using the Bioconductor package DESeq2 (Love et al., 2014) with the threshold of false discovery rate (FDR) at 0.05 level. Expression values were transformed using DESeq2's regularized log transformation (rlog) before visualization using principal component analysis (PCA). A schematic of the overall RNA-seq protocol is shown in Figure 5-1.

Chromatin Immunoprecipitation (ChIP), library preparation, and sequencing (ChIP-seq) were performed by Active Motif Services (Carlsbad, CA). In brief, genomic DNA regions of interest from sample chromatin were isolated using anti-PGR antibody and anti-ESR1 (sc-7208, Santa Cruz Biotechnology). Illumina sequencing libraries were prepared from the ChIP and Input DNAs and sequenced on Illumina's NextSeq 500 (75 nt reads, single end).

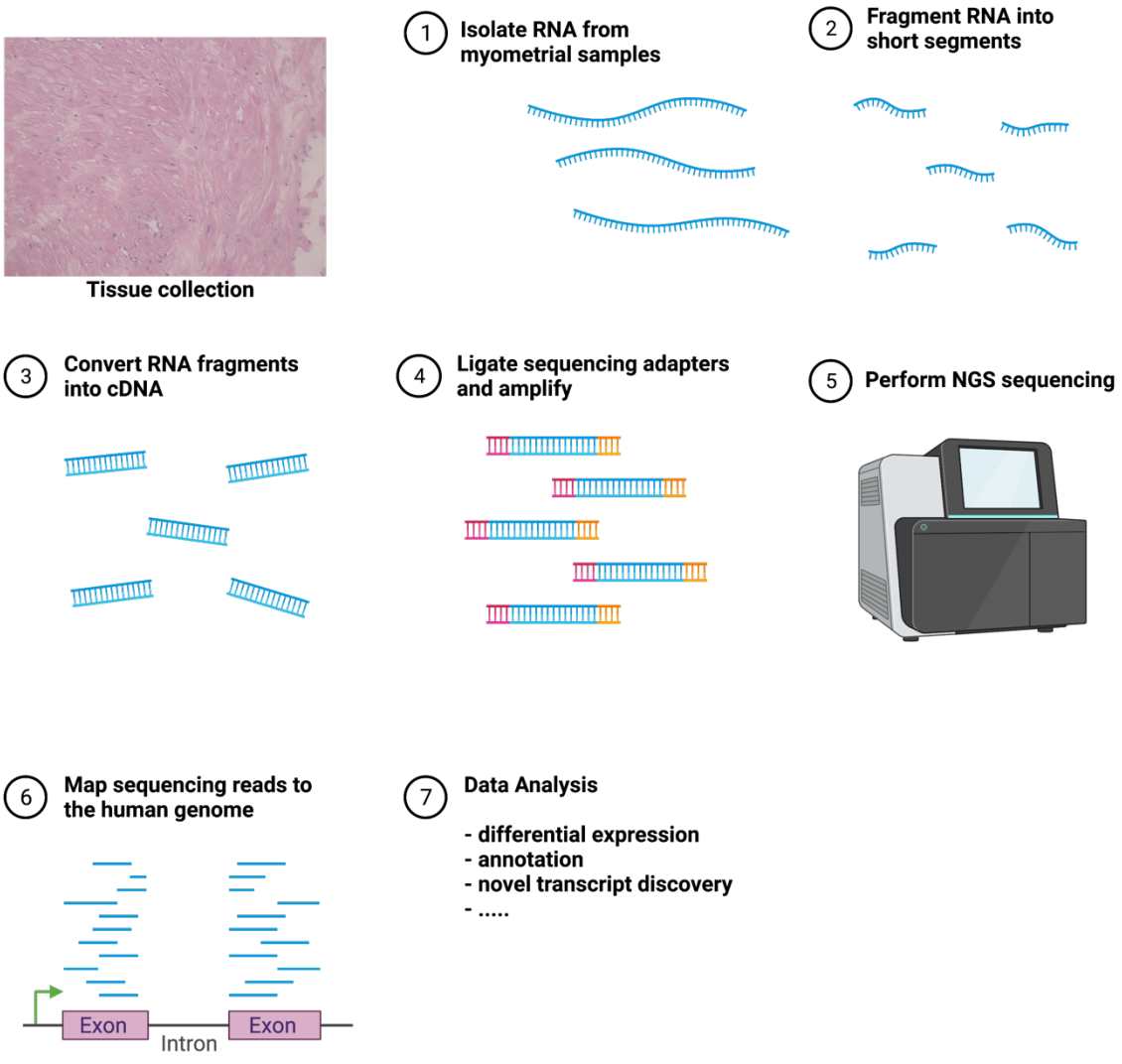


Figure 5-1

Description of steps of RNA-seq protocol. It involves (1) extracting total RNA from the tissues, (2) fragmenting that RNA, (3) performed reverse transcription to make cDNA libraries and (4) ligating adapters, which permit sequencing, to the ends of the DNA and (5) sequencing these fragments. (6) After sequencing the reads are mapped to the human genome to identify the expression of genes within samples. Finally, (7) differential expression analysis and other downstream analyses are performed

ChIP-seq Data Analysis

The 75-nt single-end sequence reads were mapped to the GR38 human genome assembly using Bowtie2 with the in-house script and alignment information for each read stored in the BAM format (Langmead et al., 2012). Peak calling in the ChIP-seq data for each sample was performed using HOMER (Heinz et al., 2010): findPeaks (-factor) for PGR/ESR1 ChIP-Seq, and findPeaks (-histone) for H3K27ac and H3K4me ChIP-seq (Figure 5-2). BED files were constructed from the peaks identified by using HOMER (pos2bed function). The BAM and BED files from replicates were used as input to the Bioconductor package DiffBind (Ross-Innes et al., 2012) to identify merged intervals of peaks and consensus overlapping regions across all replicates in the TIL and TNIL groups.

ChIPseeker (Yu et al., 2015) was used to annotate the consensus regions of the TIL and TNIL groups. Differential bound sites in the consensus regions were detected using DiffBind which combines the results from DESeq2 and edgeR ($P<0.05$) based on the read counts. The quality of the differentially bound sites was examined by PCA. In the PGR/ESR1 ChIP-seq data, differentially bound sites with a loading value magnitude greater than 0.005 in the second principal component were considered noisy sites and removed from the set of differential sites. Fisher's Exact test was used to access the significance enrichment or deviation of ChIP-seq signal in each annotated region across the differentially bound sites from the expected signal distribution across the entire set of consensus sites.

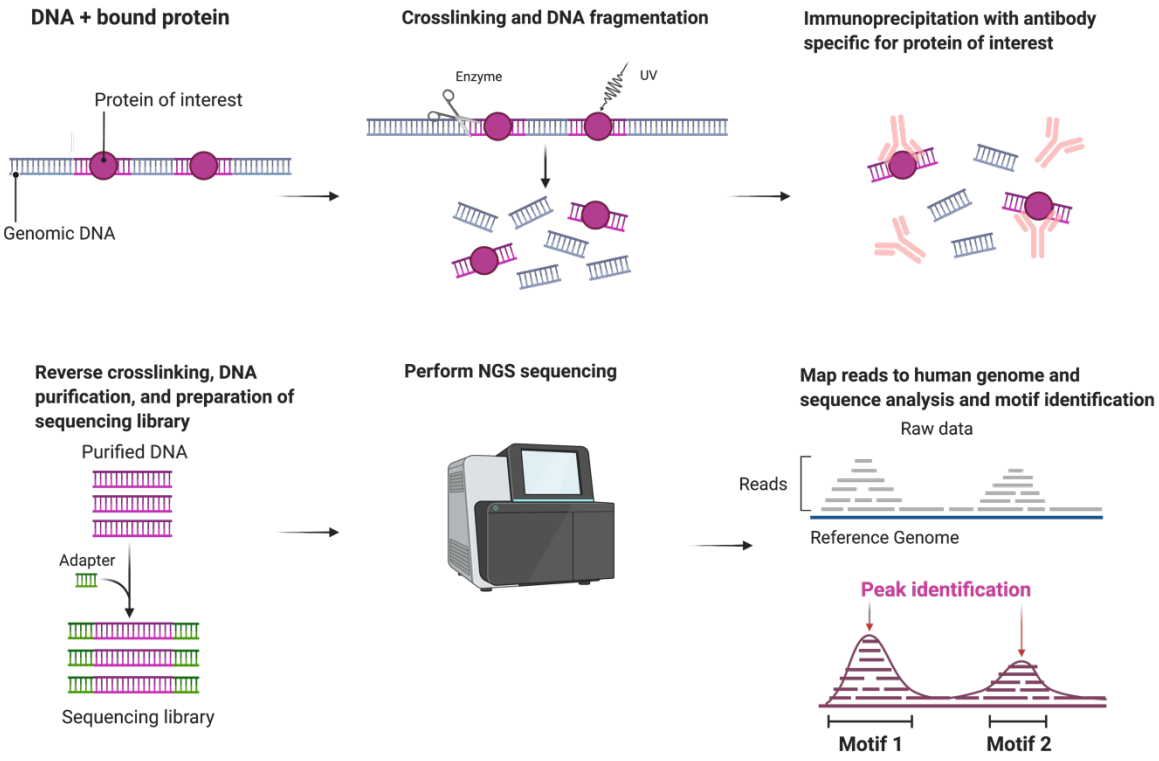


Figure 5-2

Chromatin is extracted from tissue (or cells) this contains, RNA, protein, and DNA. Next, cross-linking will keep proteins bound to DNA, then fragmentation creates fragments of DNA bound and unbound by protein. Following fragmentation, immunoprecipitation is performed with antibodies for the protein of interest. This results in pulldown of DNA fragments bound by the proteins. After pulldown, reverse crosslinking allows for removal of the proteins from the DNA, resulting in naked DNA fragments. These DNA fragments are then purified and prepared for sequencing by creating a DNA library ligated with adapters. These libraries are mapped to the sequence of the human genome and peaks identified and downstream analyses such as motif identification can be performed.

Motif analyses in the consensus regions and in the peak regions of each replicate were performed using HOMER (findMotifsGenome.pl) with the region size equal to the size of the peak (-size given). Fluff (Georgiou et al., 2016) was used to generate the profile plots of ChIP-seq peaks using the BED files of the consensus overlapping regions from DiffBind and the individual samples' BAM files (not shown).

Integrative Analysis of RNA-seq and ChIP-seq Data

CistromeGO (Li et al., 2019) was used to integrate RNA-seq with PGR and ESR1 ChIP-seq. The output files from DESeq2 on RNA-seq data and the ChIP-seq consensus regions in BED format using the negative log p-values for the score column was used as input. Cistrome-GO first computes 1) a gene ranking (R_{DE}) in RNA-seq data determined by differential expression level (LogFoldChange) multiplied by the negative Log of adjusted P-values between TIL and TNIL, and 2) a gene ranking (R_{RP}) in a ChIP-seq data set by the adjusted regulatory potential (RP) score, where RP score is defined by $s_g = \sum_{i=1}^{k} 2^{-\frac{d_i}{d_0}}$ with d_i the distance between the ith peak's center in the promoter and the transcription start site of a gene and d_0 a parameter with default value for promoter-type transcription factor. It then determines the aggregated gene rank by the product of the two ranks ($R_{DE} * R_{RP}$). The final list of the differentially expressed genes with at least one differential PGR binding site was determined using the criteria that 1) the expression changes between TIL and TNIL (FDR <0.05), 2) the occupancy changes of PGR/ESR1 between TIL and TNIL at the consensus overlapping sites (RPscore > 0.1) and 3) occupancy changes of at least one histone mark between TIL and TNIL within the gene in the same direction of differential gene expression.

Cell Culture

PHM1-41 cells were received from American Type Culture Collection (ATCC). Cells were cultured in DMEM (ATCC No. 30-2002) supplemented with 2 mM glutamine, 0.1 mg/mL G-418, and 10% heat-inactivated fetal bovine serum (FBS) in 37°C incubator with 5% CO_2. Media was refreshed every 2 to 3 days before passaging for experiments. To assess candidate gene regulation by P4, cells were incubated with 10^{-5} M P4 and vehicle for 24 hours before RNA extraction and complementary DNA (cDNA) synthesis for real-time quantitative PCR (RT-qPCR).

Real-time quantitative PCR (RT-qPCR)

About 0.5-1 µg of total RNA was reverse transcribed using qScript cDNA Synthesis Kit (QuantaBio, 95047-100). All TaqMan assays used for this study were purchased from ThermoScientific (Catalog No. 4331182). Results were analyzed in GraphPad Prism version 9.3.1. For analysis of RT-qPCR data, a two-tailed Welch's *t* test assuming unequal variance was used.

Chapter 6 DISCUSSION AND CONCLUSIONS

6.1 DISCUSSION

6.1.1 Discussion – Genome-wide Transcriptional Changes in Pregnant Myometrial Tissues Reveal Pathways That May Have Implications in Labor

This study demonstrates the transcriptome of TIL and TNIL myometrium highlighting the differences between the two phenotypes. Distinct clustering between the two groups was shown by PCA and over 1400 significant (FDR < 0.05) DEGs were found, contributing to the transcriptomic differences observed. Among statistically significant DEGs, calgranulin A (S100A8), calgranulin B (S100A9), IGF-binding protein (IGFBP3), Alpha-actin (ACTA1), GATA Binding Protein 3 (GATA3), and Fms-Like Tyrosine Kinase 1 (FLT1) were present, consistent with other studies that determined differentially expressed genes in the myometrium of term women in labor compared to women not in labor (Mittal et al., 2010; Stanfield et al., 2019). In addition, a meta-analysis examining transcriptional differences between term pregnant laboring and non-laboring myometrium found 126 high confidence differentially expressed genes from three datasets (Stanfield et al., 2019). Our differential expression analysis contained 46 of the 126 (Table 6-1). Remarkably, the direction of differential expression was consistent among these genes found in the present study and others (Chan et al., 2014; Mittal et al., 2010; Stanfield et al., 2019). Of those 46 genes, all but one were upregulated. Metallothionein 2A (MT2A), Purine Nucleoside Phosphorylase (PNP), Nicotinamide Phosphoribosyltransferase (NAMPT), Interleukin 1- Beta (IL1B), Triggering Receptor Expressed on Myeloid Cells 1 (TREM1), Serpin Family A Member 1 (SERPINA1), Metallothionein 1X (MT1X), Superoxide Dismutase 2 (SOD2), Solute Carrier Family 7 Member 5 (SLC7A5), Chromosome 15 Open Reading Frame 48 (C15orf48), RAS Like Estrogen Regulated Growth Inhibitor (RERG), Rho Guanine Nucleotide Exchange Factor 37 (ARHGEF37), Transcription Elongation Factor A Like 4 (TCEAL4), Calcium/Calmodulin Dependent Protein Kinase II Gamma (CAMK2G), Glycogenin 2 (GYG2), and Secernin 1

(SCRN1) were among upregulated genes. Protein phosphatase 1 regulatory subunit 3C (PPP1R3C) was the only high confidence gene downregulated between laboring and non-laboring myometrium in our dataset and others.

Gene Ontology uncovered pathways involved in muscle contraction and filament movement processes were enriched in genes highly expressed in TNIL myometrium (Figure 2-4). Many genes encoding for actins, myosin light and heavy chains, tropomyosins, calcium voltage-gated channel subunits, and integrins were enriched within the muscle contraction and actin filament pathways. Calcium supports smooth muscle contraction via binding to calmodulin and phosphorylating the myosin light chain kinase, allowing myosin to interact with actin (Figure 1-5 and Figure 1-6). Calcium sensitization is achieved by Rho/Rho-kinase signaling to inhibit dephosphorylation of the myosin light chain kinase (Figure 1-5). Because contractions are physically present at the time of collection for the TIL group, it is possible that the gene expression changes required to alter protein action have already occurred. Thus, the transcriptomic snapshot taken at tissue collection misses the timepoint in which mRNA levels affect contraction initiation in the TIL group. Inflammatory and immune responses were among the enriched pathways in genes highly expressed in TIL (Figure 2-4), consistent with previous studies using RNA-seq and microarray (Mittal et al., 2011; Stanfield et al., 2019). This is consistent with many studies in which inflammation and immune regulation are implicated in labor (Romero et al., 2014; Cappelletti et al., 2016; Goncalves et al., 2002; Gomez et al., 1995; Kim et al., 2012; Elovitz et al., 2003; Phung et al., 2022; Rajagopal et al., 2015).

Gene ID	Gene Symbol
ENSG00000163638	ADAMTS9
ENSG00000148926	ADM
ENSG00000167772	ANGPTL4
ENSG00000166825	ANPEP
ENSG00000103569	AQP9
ENSG00000125845	BMP2
ENSG00000196352	CD55
ENSG00000131873	CHSY1
ENSG00000163464	CXCR1
ENSG00000180871	CXCR2
ENSG00000211448	DIO2
ENSG00000197406	DIO3
ENSG00000157557	ETS2
ENSG00000085265	FCN1
ENSG00000171049	FPR2
ENSG00000100644	HIF1A
ENSG00000125538	IL1B
ENSG00000168685	IL7R
ENSG00000136167	LCP1
ENSG00000187116	LILRA5
ENSG00000071282	LMCD1
ENSG00000171236	LRG1
ENSG00000187193	MT1X
ENSG00000125148	MT2A
ENSG00000105835	NAMPT
ENSG00000099985	OSM
ENSG00000011422	PLAUR
ENSG00000198805	PNP
ENSG00000119938	PPP1R3C
ENSG00000148344	PTGES
ENSG00000118849	RARRES1
ENSG00000121039	RDH10
ENSG00000143333	RGS16
ENSG00000163162	RNF149
ENSG00000143546	S100A8
ENSG00000163220	S100A9
ENSG00000188404	SELL
ENSG00000197249	SERPINA1
ENSG00000116991	SIPA1L2
ENSG00000155926	SLA
ENSG00000103257	SLC7A5
ENSG00000112096	SOD2
ENSG00000123610	TNFAIP6
ENSG00000124731	TREM1
ENSG00000117143	UAP1
ENSG00000148154	UGCG

Table 6-1

List of overlapping high confidence differentially expressed genes in present work and Stanfield et al., 2019

6.1.2 Discussion – Progesterone Receptor Genome-wide Changes in Pregnant Myometrial Tissues Reveal Players in Labor

To our knowledge, this is the first analysis comparing the PGR cistrome in term pregnant laboring and non-laboring human myometrium. PGR cistromic data show differential PGR binding profiles between laboring and non-laboring myometrium (Figure 3-6). Not only is there a difference in the genomic regions in which PGR is binding, but there are also differences in the sequences in which PGR binds (Figure 3-4; Figure 3-5). Binding in TNIL was substantially different in that it was primarily in the intergenic regions and specific for only 12 motifs, with hypoxia associated motifs overrepresented (HIF1a and HIF2a) and lacking in hormone response elements. In contrast, PGR binding in TIL was broader, binding to 228 motifs including a classical progesterone response element (PRE) and concentrated in the promoter regions. However, the presence of the classical PRE present in the TIL binding sites and not in the TNIL binding sites is inconsistent with PGR occupancy in term pregnant non-laboring myometrium from a recent study (Wu et al., 2019). Additionally, intronic regions and enhancers were highly occupied by PGR in term non-laboring pregnant myometrium from the previous study, whereas in our study, mainly distal intergenic/enhancer regions were enriched in term non-laboring pregnant myometrium (58.33%).

Despite these differences, the present study found interesting motifs associated with PGR interacting with chromatin in TIL myometrium. A portion of these motifs, such as MyoD, STAT, YY1, and SMAD2, overlapped with previously reported motifs in term pregnant non-laboring myometrium (Wu et al., 2019). Interestingly, CTCF and BORIS motifs were the top enriched motifs found in TIL PGR binding sites. CTCF is involved in forming long-range-chromatin loops and acts as insulators of transcriptional activity (Wang et al., 2012). The ETS family of

transcription factors (ELK4, ELK1, ELF1, FLI1) play critical roles in various biological processes including tissue remodeling (Dittmer et al., 2003; Hsu et al., 2014; Oikawa et al., 2003).

Differential binding analysis for PGR uncovered pathways involved in cAMP signaling and cardiac muscle cell membrane repolarization were uniquely enriched in PGR binding regions found gained in labor (Figure 3-7). Genes in the cAMP signaling pathway included phosphodiesterase 4A (PDE4A) and phosphodiesterase 10A (PDE10A), a class of enzymes involved in the regulation of intracellular cAMP and cGMP levels important for smooth muscle contractility. Selective inhibition of PDE4 has been implemented in smooth muscle relaxation (Buhimschi et al., 2004; Mehats et al., 2007; Oger et al., 2004). Cell membrane repolarization, negative regulation of CREB activity, and negative regulation of interleukin-2 production were also uniquely enriched for genes found to have enhanced PGR binding in TIL myometrial tissue (Figure 3-7).

In contrast, integrin activation, adhesion related pathways, and cytoskeleton transport were uniquely enriched for genes that lost PGR binding in labor (Figure 3-7). These differences suggests that PGR candidate genes in these unique pathways may be important for labor status. It has been reported that PGR isoforms and PGR-A:PGR-B ratio are crucial for the switch from a quiescent phenotype to contractile phenotype (Mesiano et al., 2002; Merlino et al., 2007; Nadeem et al., 2016; Nadeem et al., 2017; Patel et al., 2018; Vegeto et al., 1993). However, given that the PGR ChIP-seq antibody detects all isoforms of PGR, there is no indication of whether isoform differences contribute to the differential binding seen, limiting our study. Thus, further investigation on the role of the isoforms in human tissue is needed. Overall, the PGR cistromic data sheds light on the targeted pathways and genes in pregnant myometrium that may contribute to the initiation of labor.

Integrative analysis using CistromeGO and differential histone ChIP-seq revealed ATP11A, CBX7, and TNS1 as possible P4 responsive quiescent/contraction associated genes. CBX7 is the gene that encodes the Chromobox 7 protein, a component of a polycomb group, critical for transcriptional repression of many genes, such as the HOX gene family (Yap et al., 2010). So, there are many implications for effects on canonical PRC1 complex in biological processes. Interestingly, the expression of CBX7 is downregulated in labor, and inversely upregulated in the non-laboring group, and HOXA1 motifs were enriched in the PGR binding sites from TNIL. Taken together, this data supports a relationship between HOX genes and PGR in non-laboring myometrium.

ATP11A is an ATPase that catalyzes the hydrolysis of ATP coupled to transporting phosphatidylserines. This phospholipid transporter protein with ATPase activity stimulates calcium influx and Rho GTPase signaling, leading to the assembly of myosin fibers and myotube formation in myoblasts (Tsuchiya et al., 2018). The upregulation of ATP11A in labor may contribute to the increased assembly of myosin fibers required for myosin and actin cross-bridges. Finally, TNS1 has been shown to be essential for myofibroblast differentiation (Bernau et al., 2017). Additionally, it is involved in tethering actin to integrin and forming focal adhesions that are required for actin-based traction. Without focal adhesions vascular smooth muscle cell contractility is impaired (Ribeiro-Silva et al., 2021). Therefore, downregulation of TNS1 may contribute to affecting the myometrial contractile potential.

Validation of candidate gene hormone responsiveness *in vitro* using the pregnant myometrial cell line (PHM1-41) yielded conflicting results from our *in vitro* analysis. Integration data from tissue revealed PGR as a repressor of CBX7 but an activator *in vitro*. Although tissue culture is a great model for the *in vivo* environment, previous studies demonstrated that

progesterone/PGR signaling needs the tissue intact to fulfill its appropriate genomic function (Ikhena et al., 2018; Tanos et al., 2013). Thus, the *in vitro* model cannot fully recapitulate the *in vivo* condition. Additionally, this *in vitro* model does not assess functional PGR isoforms. The regulation of candidate genes by PGR isoform should be addressed in future *in vitro* studies using models that can manipulate isoform levels.

6.1.3 Discussion – Unwrapping ESR1 Binding Maps in Pregnant Myometrial Tissues to Reveal Potential Contributors in Parturition

ESR1 ChIP-seq in human tissue has been performed primarily on female reproductive cancers tumors, such as breast cancer, and, to our knowledge, not in pregnant human myometrium (Jansen et al., 2013; Ross-Innes et al., 2012; Severson et al., 2018; Tian et al., 2017). ESR1 mapping in term pregnant myometrial tissues revealed no differences in ESR1 binding patterns (Figure 4-4; Figure 4-5). These results suggest that ESR1 may not strongly influence the transcriptional differences seen in labor. Despite these results, 1499 genes were differentially expressed in labor and differentially bound by ESR1, dependent upon labor status.

Remarkably, calcium signaling and stress-activated protein kinase signaling were among the pathways associated with genes found with a loss of ESR1 binding in labor. Previous studies found a relationship between MAPK activation, calcium, and smooth muscle contraction (Dessy et al., 1998; Gerthoffer et al., 2003; Takahashi et al., 1998). ESR1 binding sites gained in labor were mostly enriched for neuronal pathways and, strikingly, the establishment of chromosome localization. This suggests that the slight differences between TIL and TNIL may have specific roles in the tissue.

The role of estrogen and inflammation has been studied in other types of smooth muscle cells. Estradiol treatment of vascular smooth muscle cells results in inhibition of inflammatory

genes, IL-6, and IL-1 (Kikuchi et al., 2000). Furthermore, estrogen at pregnancy levels inhibits IL-1B and NF-kappa-B activation in rat vascular smooth muscle (Sharma et al., 2001). Thus, it is not surprising that the estrogen-responsive DEGs in labor have a role in inflammatory processes such as interaction with I-kappa-B, leukocyte migration, and lipopolysaccharide-mediated signaling (Figure 4-8). In comparison, DEGs with gained sites in labor were enriched for muscle contraction, cytoskeleton organization, cell adhesion, and wound healing. This observation can be interpreted as ESR1 having distinct roles in pregnant myometrium, focusing on regulating CAPs for muscle contraction, wound healing, and organization of the dynamic tissue changes associated with birth in laboring myometrium.

Although the ESR1 candidate genes are not explored in the present work, they should not be overlooked (Table 4-1). The gene CACNA1D encodes an L-type calcium channel, which mediates the entry of calcium into the cell, an essential aspect of smooth muscle contractility (Figure 1-5). Trio Rho Guanine Nucleotide Exchange Factor (TRIO) is a GTPase that promotes the reorganization of the actin cytoskeleton. As previously mentioned, cytoskeletal reorganization is important for pregnant myometrial function, such as focal adhesion formation (Chapter 6.1.2). Mechanical stretch of myometrial smooth muscle cells is critical for myometrial contractility, and it is posited that mechanical stretch partnership with hormones initiates labor (Garfield et al., 2007). Vinculin (VCL) is strongly expressed at focal adhesion sites in human myometrial cells, becoming another strong candidate for further implication in labor (Yu and Lopez Bernal 1998).

6.2 Future Directions

One drawback of the current thesis work is the exclusion of preterm laboring and non-laboring myometrium. Other studies have reported that the etiology of preterm birth is distinct from term birth (Gibb et al., 2002; Phung et al., 2022). Thus, the inclusion of preterm laboring

myometrium should be included and used in candidate gene validation and mechanistic experiments.

To truly unearth key players that modulate myometrial contractility, myometrial tissue explants are great *ex vivo* models. They closely resemble *in vivo* gene and protein expression more than cell culture models (Georgiou et al., 2016; Arrowsmith et al., 2018). Nevertheless, ethical and collection restraints pose as an obstacle with this model system. Overall, human tissue that allows for testing potential tocolytic agents is the best system for exploring the pathways involved in myometrial contractility that leads to labor. Thus, to validate the importance of these candidate genes, validation studies in an ex vivo model would be ideal (Figure 6-1). This system allows for the assessment of tissue contraction performance and the direct effect of different agents on the parameters of contraction to be measured, including contraction force (strength), frequency, and duration, as well as the integration of these values, to generate an index of the total work done.

This experiment can also serve as a drug discovery experiment. Strips of pregnant human myometrium would be dissected, trimmed, and mounted to a tissue bath containing saline (about 2mm x 8mm x 1 mm wide, long, and thick, respectively), where spontaneous contraction can be recorded with a transducer. The tissue would be stimulated with potential drugs that target the genes or pathways of interest in addition to positive control treatments, such as oxytocin, and negative controls, such as atosiban (OXT antagonist) and saline. Spontaneous and drug-induced contractility would then be recorded and analyzed. Western blot or qPCR can be used on the tissue strips to determine protein and gene expression of candidate genes to validate drug-induced gene expression changes. These results would further support the candidate gene's role in myometrial relaxation/contractility as recorded from the experiment.

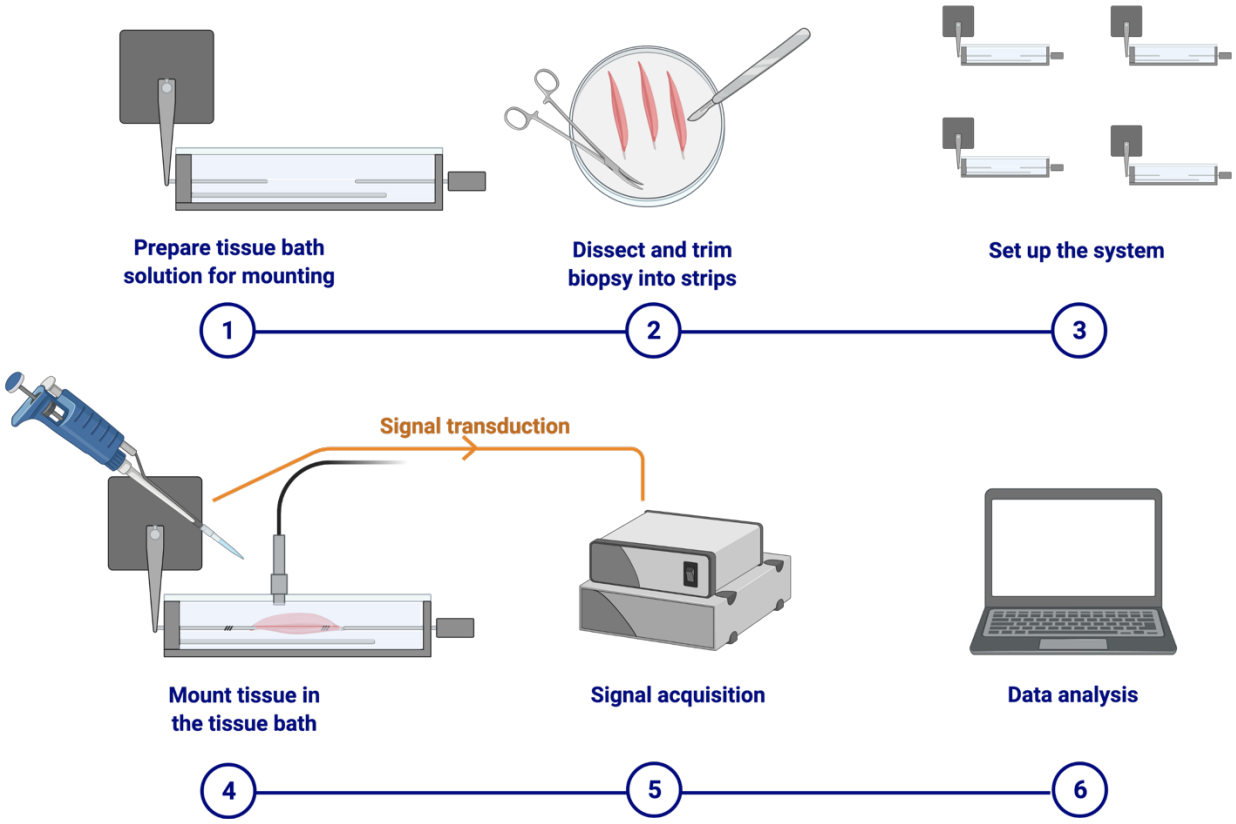


Figure 6-1

Schematic of myometrial ex vivo experiment that would provide further insight into the therapeutic potential of candidate genes in myometrial contractility associated with the labor cascade. This (1) contraction apparatus is set up with physiological saline to record contractility Strips of pregnant human myometrium would be (2) dissected and trimmed. This apparatus allows one or (3) multiple strips to be used for experiments. Once the tissue is properly prepared, it will be (4) mounted to the tissue bath, where spontaneous contraction and drug-induced contractility can be recorded with a (5) transducer and (6) analyzed.

6.3 Conclusion

This work hypothesized that progesterone receptors and estrogen receptors play critical roles in human labor by regulating labor-associated genes. In summary, we have found that PGR interacting partners in contractile myometrium in labor are involved in chromatin remodeling and epigenetic regulation. These transcription factors and pathways working with or under the control of PGR are manipulating inflammatory pathways, cAMP signaling, and cell membrane polarization pathways. This multifaceted process leads to the switch from quiescence to contraction in pregnant human myometrium (Figure 6-2).

This work provides a critical genome-wide database for future studies of parturition in human myometrium. It sets a foundation for research involving PGR and ESR1 (Chapter 3; Chapter 4) in the human labor cascade and possible target genes and pathways for therapeutic intervention in myometrial contraction and is the only study, to date, to explore chromatin interactions with PGR and ESR1 in term pregnant human myometrium. Furthermore, this is the most novel and advanced RNA-seq in term pregnant human myometrium that explores the transcriptomic profiles between laboring and non-laboring phenotypes (Chapter 2).

These studies have generated two papers, to date, in review. I have presented my work outside of the Northwestern community at various biomedical and clinical international conferences. Inside the Northwestern community, this work was presented at research seminars and Research Day. Lastly, I was inducted into the Edward A. Bouchet Graduate Honor Society for my research merit, leadership, advocacy, character, and exemplary service (2020).

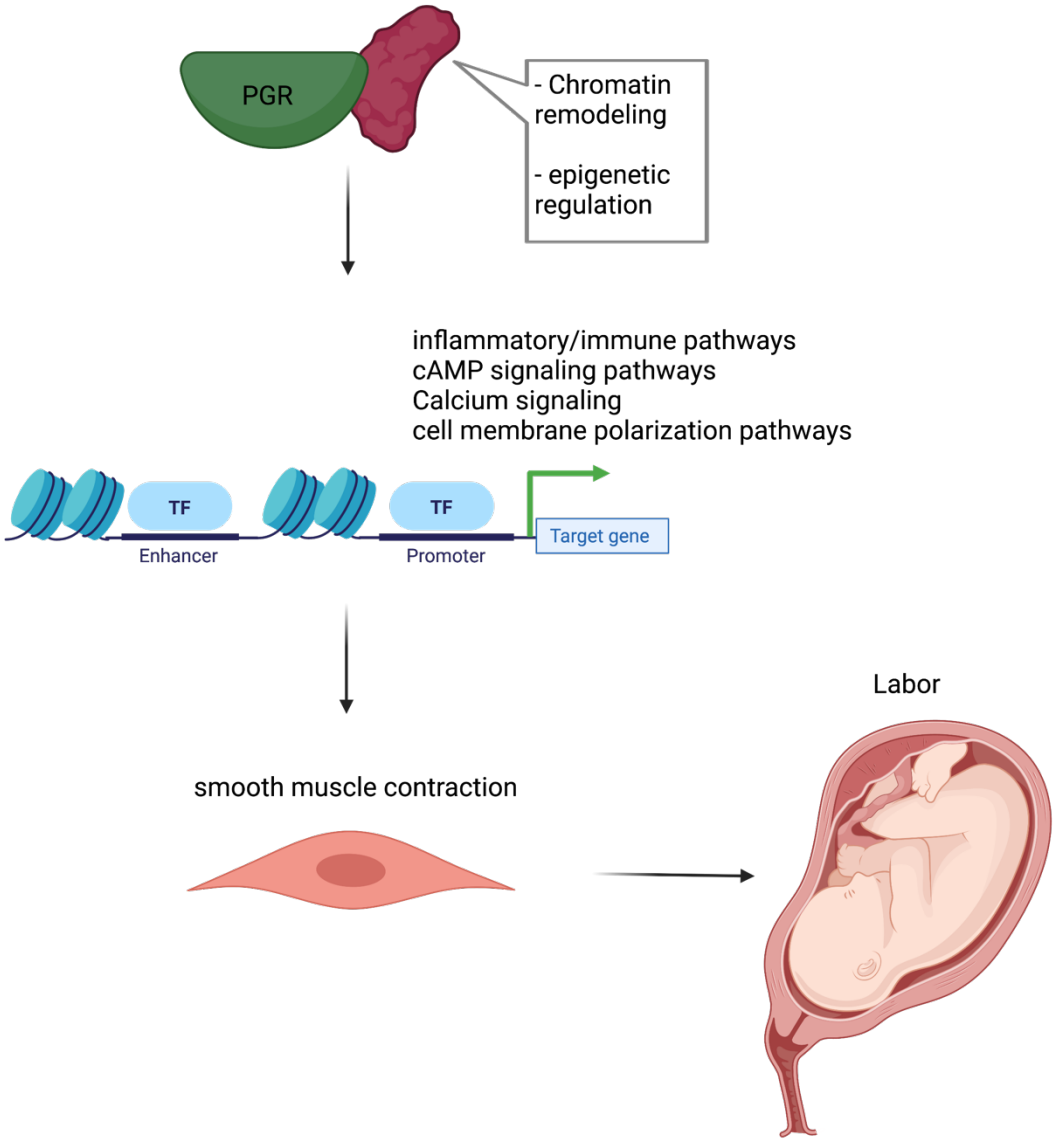


Figure 6-2

PGR and its interacting partners, including proteins involved in chromatin remodeling and epigenetic regulation, influence labor associated pathways in myometrial cells such as inflammation, cell membrane polarization, cAMP, and calcium signaling. These pathways influence the transition of myometrial smooth muscle cells to a contractile state, leading to labor.

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