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Fetal-to-Maternal Signaling in the Timing of Birth

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Abstract

Preterm birth remains the major cause of neonatal morbidity and mortality throughout the world. This is due, in part, to our incomplete understanding of the mechanisms that underlie the maintenance of pregnancy and the initiation of parturition at term. In this article, we review our current knowledge of the complex, interrelated and concerted mechanisms whereby progesterone maintains myometrial quiescence throughout most of pregnancy, as well as those that mediate the upregulation of the inflammatory response and decline in progesterone receptor function leading to parturition. Herein, we review findings that demonstrate a role of the fetus in the timing of birth. Specifically, we focus on our own studies indicating that maturation of the fetal lung and enhanced secretion of the surfactant components, surfactant protein A (SP-A), and the potent inflammatory glycerophospholipid, platelet-activating factor (PAF) initiate a signaling cascade culminating in parturition. Our studies suggest an essential role of steroid receptor coactivators, SRC-1 and SRC-2, which activate expression of genes encoding SP-A and LPCAT1. LPCAT1 is a key enzyme in the synthesis of PAF, as well as DPPC, a highly surface-active glycerophospholipid component of surfactant. Thus, we describe a novel pathway through which the fetus contributes to the initiation of labor by signaling the mother when its lungs have achieved sufficient maturity for survival in an aerobic environment.

Keywords

Pregnancy; parturition; progesterone; NF- κ B; fetal lung; surfactant protein A; platelet-activating factor

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1. Introduction

Preterm birth (<37 weeks gestation) is the leading cause of infant mortality during the first four weeks of life throughout the world [1]. The highest rates of preterm birth are found in parts of Africa and in North America. In the United States, the incidence of preterm birth has increased steadily over the past several decades and has recently plateaued at ~11.5% of all live births. Moreover, there remain significant and persistent racial disparities in preterm birth rates. While the prematurity rate among white infants is ~11%, close to 18.0% of births among black infants are preterm. The underlying factors contributing to this racial disparity remain unclear [2]. Whereas, infection with associated inflammation of the fetal membranes likely contributes an important stimulus for preterm labor [3,4], signaling molecules that promote the inflammatory response leading to labor at term are less certain. There is increasing evidence to suggest that the fetus generates signals that contribute to the initiation of labor. In this mini-review, we will consider evidence to suggest that the fetus produces signals leading to labor at term. We first will consider the fetal tissues and signaling molecules proposed to serve a role in the initiation of parturition at term and then focus on our own studies which strongly suggest that these signaling molecules arise from the fetal lung.

2. Progesterone (P₄) acting through nuclear progesterone receptor (PR) maintains myometrial quiescence throughout most of pregnancy

Myometrial quiescence throughout most of pregnancy is mediated by increased circulating levels of P₄, produced by placenta and/or the ovarian corpus luteum, depending upon the species. Progesterone blocks myometrial contractility through its binding to two nuclear receptor isoforms, PR-A (94 kDa) and PR-B (114 kDa), which are the products of a single gene [5,6]. As shown in Figure 1 (left panel), the mechanisms whereby P₄/PR maintains uterine quiescence are multiple and overlapping. For example, P₄/PR can exert a pronounced anti-inflammatory effect, by inhibiting activation/DNA binding of the pro-inflammatory transcription factors, nuclear factor κ B (NF- κ B) [7] and activating protein 1 (AP-1) [8]. P₄/PR also can block induction of the proinflammatory response by upregulating expression of the NF- κ B inhibitor, I κ B α [7], and the mitogen-activated protein kinase (MAPK) inhibitor, MAPK phosphatase-1/dual specificity phosphatase 1 (MKP-1/DUSP1) [9]. Moreover, P₄/PR also maintains myometrial quiescence by increasing expression of the transcriptional inhibitor, ZEB1, which binds to the promoters of the contractile genes, gap junction protein connexin-43 (*CX43/GJA1*) and oxytocin receptor (*OXTR*), to suppress their expression throughout most of pregnancy [10,11].

3. Inflammatory signaling and the initiation of term and preterm labor

Labor, both term and preterm, is accompanied by an inflammatory response. Increased levels of proinflammatory cytokines are found in amniotic fluid [12] and the myometrium, cervix, and fetal membranes are infiltrated by immune cells [13–15], which secrete proinflammatory cytokines and chemokines [3] (Fig. 1, **right panel**). This promotes an inflammatory signaling cascade that results in activation of NF- κ B and other proinflammatory transcription factors (*e.g.* AP-1) in myometrium [14,16], cervix and fetal

membranes [17–19]. Activated NF- κ B and AP-1 increase expression of genes that promote myometrial contractility, including the prostaglandin F₂ α receptor [20], *CX43* [21], *OXTR* [22] and cyclooxygenase-2 (*PGHS2*, COX-2) [23], which catalyzes the production of contractile prostaglandins [24–26]. In preterm labor, intra-amniotic infection associated with chorioamnionitis may provide the stimulus for increased amniotic fluid interleukins and inflammatory cell migration [27]. On the other hand, the inflammatory response leading to labor at term is triggered by increased mechanical stretch [28,29] imposed by the growing conceptus, as well as by fetal signaling molecules produced in increasing amounts toward term [14,30–35] (Fig. 1), which are described in *Section 4*.

The increased inflammatory response that occurs toward term also is caused by a relative increase in E₂/ER α signaling and decline in P₄/PR function (Fig. 1, **right panel**). The increase in circulating estradiol-17 β (E₂) [36,37] and/or increased estrogen receptor α (ER α) activity [38,39] induce immune cell migration into the uterus and antagonize the anti-inflammatory actions of P₄/PR [39,40]. Furthermore, ER α activation directly enhances transcription of *OXTR* [41], *CX43* [42] and *COX-2* [39]. Importantly, the enhanced inflammatory response further impairs PR function in myometrium through upregulation of NF- κ B and AP-1, which directly interact with PR to impair its transcriptional activity, causes a decline in PR coactivators [43,44], and increases the expression of truncated PR-A [39,45] and PR-C [16] isoforms relative to full-length PR-B (Fig. 1). The relative increase in E₂/ER α activity and decline in P₄/PR function near term also cause downregulation of myometrial ZEB1, resulting in upregulation of the miR-200 family [10], which directly targets STAT5B, causing the induction of 20 α -hydroxysteroid dehydrogenase, which increases the local metabolism of P₄ to inactive products [46].

4. Fetal signals leading to the initiation of parturition

There is accumulating evidence that the fetus secretes hormonal signals that contribute to the initiation of labor at term. Described below are several of the signaling molecules and the fetal tissues from which they arise in a number of different animal models.

4.1 Fetal adrenal cortisol production

During late gestation in sheep, maturation of the hypothalamic-pituitary-adrenal axis promotes increased cortisol production by the fetal adrenal [47]. The increased fetal cortisol can enhance production of surfactant components by the fetal lung [48], which as discussed below, may serve as important fetal signals for the initiation of labor. The increased cortisol also stimulates expression of placental COX-2 and production of prostaglandins, which enhance 17 α -hydroxylase/17,20 lyase (CYP17) and synthesis of C₁₉-steroids, which are metabolized to estrogens by placental aromatase P450 (CYP19). The increased estrogens induce immune cell migration into the uterus, antagonize anti-inflammatory actions of P₄/PR [39,40] and turn on proinflammatory and contractile genes [24–26,39,41,42], as described above.

4.2 Placental corticotropin-releasing hormone

CRH uniquely produced by the human placenta near term, has been implicated as a fetal signal for labor [49–51]. Unlike sheep, the human placenta does not express CYP17 and therefore cannot convert progestins to C₁₉-steroids, which instead, are produced in exceedingly high amounts by the fetal adrenals [52]. Increased placental CRH is suggested to stimulate adrenocorticotrophin (ACTH) secretion by the fetal pituitary, which enhances production of cortisol and C₁₉-steroids by the fetal adrenals. C₁₉-steroids can be metabolized to estrogens in the placenta, which enhance the myometrial inflammatory response and contractile gene expression, as described above. Notably, CRH mRNA also is developmentally upregulated in fetal mouse lung bronchiolar epithelium between 13.5 and 17.5 dpc and is then diminished at 18.5 dpc [53]. This increase in lung CRH precedes the developmental induction of fetal lung SPA expression. Accordingly, in *CRH* null mouse fetuses, lung maturation and induction of *SP-A* expression were impaired [54]. This could be due to the lack of local actions of CRH within the fetal lung, or to the effects of diminished fetal adrenal cortisol production on lung maturation and the production of surfactant components.

4.3 Surfactant lipids and proteins produced by the fetal lung serve as signals for the initiation of parturition

Maturation of the fetal lung and increased production of pulmonary surfactant components has been proposed to provide signals for the initiation of parturition [14,33,35,55,56]. Surfactant synthesis by alveolar type II cells is initiated in the fetal lung after ~80% of gestation is complete and is secreted into amniotic fluid. After birth, surfactant functions to reduce alveolar surface tension and is essential for air breathing.

Dipalmitoylphosphatidylcholine (DPPC), the major glycerophospholipid surfactant component, is highly surface-active. Proteins comprise ~10% of surfactant composition. There are four surfactant proteins, SP-A, SP-B, SP-C and SP-D [57,58]. SP-B and SP-C are highly lipophilic proteins that act with surfactant glycerophospholipids to reduce alveolar surface tension [57,59]; SP-A and SP-D are collectins that function as part of the innate immune system [60]. In that capacity, they function within the alveolus to enhance uptake of variety of microbial pathogens by macrophages [58,60,61].

The first evidence that fetal lung surfactant could promote an inflammatory response within fetal membranes came from studies of Lopez-Bernal and colleagues [55] who found that surfactant from human amniotic fluid stimulated prostaglandin synthesis in human amnion discs. They proposed that surfactant phospholipids within amniotic fluid provided a source of arachidonic acid for prostaglandin synthesis. In studies of human amnion cells, others reported that a substance in amniotic fluid enhanced PGE₂ production [32]. Neither the identity nor the source of this substance was determined, although it was postulated to be produced by the fetal kidney and secreted into amniotic fluid via the urine. Subsequently, it was discovered that platelet-activating factor (PAF), a potent proinflammatory phospholipid known to be involved in hypersensitivity reactions, was secreted into amniotic fluid with fetal lung surfactant near term [62]. It was suggested that PAF of fetal lung origin may promote myometrial contractility.

4.4 Surfactant protein A produced by the fetal lung near term serves as an important signal for the initiation of parturition

As mentioned, SP-A is a C-type lectin (collectin) that acts postnatally within the lung alveolus to enhance macrophage function to engulf microorganisms [60,61,63,64]. During late gestation, SP-A produced by the fetal lung in concert with surfactant glycerophospholipids serves as an excellent marker of lung maturation and type II cell differentiation. In the fetal mouse, lung SP-A expression and secretion into amniotic fluid are induced at 17.5 dpc and continue to increase toward term (19 dpc) [14,65,66]. This was found to be associated with increased IL-1 β expression in amniotic fluid macrophages, their migration to the maternal uterus and activation of uterine NF- κ B [14].

To directly assess the capacity of SP-A to initiate labor *in vivo*, parallel groups of mice were injected intraamniotically within the right uterine horn with purified SP-A or with an SP-A-depleted preparation, as control [14]. The majority of mice injected with SP-A at 15.5 dpc delivered prematurely at 16.5–17.5 dpc from the injected horn only. Migration of fetal macrophages and activation of NF- κ B within the SP-A-injected uterine horn were detected within 4.5 h. Moreover, intraamniotic injection of an SP-A antibody resulted in a 24 h delay in labor (20 dpc) [14].

In light of the potential role of SP-A in the initiation of parturition, its structural and functional relatedness to SP-D, and the known SP-A and SP-D interactions with Toll-like receptors (TLR) [67–72], it was of interest to define the functional roles of these endogenous surfactant proteins and their putative receptors using gene targeted mice. The timing of parturition was assessed during first and second pregnancies in C57BL/6 WT mice and in mice homozygous null for *SP-A*, *SP-D* and doubly deficient in *SP-A* and *SP-D*. *SP-A*^{−/−} and *SP-A*^{−/−}/*SP-D*^{−/−} female mice bred to genetically-like males delivered normally at term during first pregnancies. However, during second pregnancies, *SP-A* and *SP-A/SP-D* null females bred to genetically-like males manifested a ~12 h delay in parturition [35] (Fig. 2A, B). While the delay did not reach statistical significance for the *SP-A* gene targeted mice ($p = 0.07$), a similar time delay in parturition in the *SP-A/SP-D* dKO mice was highly significant. We reasoned that the normal timing of parturition observed in the *SP-A* deficient and the *SP-A/D* doubly deficient mice in first pregnancies was potentially due to multifactorial regulation of parturition timing and the dominant role of uterine stretch as a signal for parturition [29,73,74] in the non-adapted uterus. However, in subsequent pregnancies, the prior mechanical adaptation of the uterus to stretch, resulting in increased elasticity [75] may allow other signals (*e.g.* surfactant proteins) to play a more significant signaling role. Myometrium of *SP-A/D*^{−/−} females expressed significantly lower levels of IL-1 β , IL-6, *Cx43* and *Oxtr* at 18.5 dpc compared to WT [35] (Fig. 2C, D). *TLR2*^{−/−} females manifested a significant (~12 h) ($P < 0.001$) delay in timing of parturition compared to WT, as well as reduced expression of *Cx43* and M ϕ marker, F4/80, at 18.5 dpc during first pregnancies [35]. F4/80⁺ AF M ϕ s from *TLR2*^{−/−} and *SP-A/D*^{−/−} mice expressed significantly lower levels of both pro-inflammatory and anti-inflammatory activation markers (*e.g.* IL-1 β , IL-6, Arg 1, Ym1) compared to gestation-matched WT AF M ϕ s [35]. These findings suggest that the pulmonary collectins acting via TLR2 serve a modulatory role in the timing of labor; their relative impact may be dependent upon parity.

4.5 Steroid receptor coactivator (SRC)-1 and SRC-2 serve key roles in fetal-to-maternal signaling leading to parturition

In studies using transgenic mice and transfected type II cells, we observed that a 300-bp region upstream of the rabbit [76–79] and human [80–86] *SP-A* genes is critical for lung cell-specific, developmental and cAMP/IL-1 β -induced expression (Fig. 3). Steroid receptor coactivators, SRC-1 and SRC-2, serve important roles in transcriptional upregulation of *SP-A* gene expression in fetal lung type II cells [85,87]. Endogenous SRC-1 in human fetal lung (HFL) type II cells was recruited to thyroid transcription factor-1 (TTF-1/Nkx2.1) and NF- κ B bound to the *hSP-A* promoter upon cAMP and interleukin-1 (IL-1) stimulation of *SP-A* expression [87,88]. cAMP also enhanced estrogen-related receptor α (ERR α) transcriptional activity at the *SP-A* promoter by increasing its interaction with protein kinase A and SRC-2 [85]. Recruitment of the histone acetylases, CREB-binding protein (CBP) and p300, by SRC-1/SRC-2 resulted in the local acetylation of histones and an opening of chromatin structure [87] (Fig. 3).

SRCs are members of the p160 coactivator family, comprised of: SRC-1 (NCoA-1, ERAP160, ERAP140), SRC-2 (NCoA-2, GRIP-1, TIF-2) and SRC-3 (AIB1, TRAM-1, RAC3, p/CIP, ACTR) [89]. Whereas SRCs do not bind to DNA directly, they upregulate transcriptional activities of nuclear receptors and other transcription factors by facilitating their interactions, and by recruiting coregulators with histone modifying activities [89,90]. In this manner they can alter chromatin structure and cause transcriptional activation or repression [91].

In studies using *Src-1*, *Src-2* and *Src-3* gene-targeted mice, different reproductive phenotypes were observed [90]. Whereas *Src-1*^{-/-} mice were fertile, females exhibited reduced stimulation of uterine growth in response to estradiol-17 β , and a decreased uterine decidual response to P₄ [92]. In males, *Src-2* deficiency resulted in infertility due to impaired spermatogenesis [93], while females manifested placental growth defects and sub-fertility [94]. Remarkably, mice that were double KO (dKO) for *Src-1* and *Src-2* died at birth of atelectasis [95], suggesting a deficiency in lung surfactant production. This was intriguing in consideration of our previous findings concerning the roles of SRC-1 and SRC-2 in SP-A expression.

To further study these mice, we bred *Src-1*/*Src-2* doubly heterozygous females (*Src-1*^{+/-}/*Src-2*^{+/-}, *Src-1/2 dhet*) with genetically like males. To our surprise, they manifested a pronounced delay in parturition timing (~38 h delay) (Fig. 4A, B) [33]. Parturition timing was normal in *Src-1*-KO males bred to *Src-1*-KO females and in *Src-2*^{+/-} (*Src-2*-het) females bred to *Src-2*-het males, suggesting that Src-1/Src-2 double-deficiency mediated the delay in parturition. Remarkably, when we crossed wild-type (WT) females with *Src-1*/*Src-2* doubly deficient males (*Src-1/2 dhet* or *Src-1*-KO/*Src-2* het) an equivalent (~38 h) delay in parturition timing also was evident [33] (Fig. 4B). This demonstrates that the Src-1/2 deficiency of the fetuses delays the timing of birth. Moreover, the delay in parturition was highly correlated with the proportion of fetuses that were doubly-deficient in *Src-1* and *Src-2* (Fig. 4C). In light of our prior work on SPA, we analyzed SP-A in lungs and amniotic fluid surrounding fetuses from *Src-1/2 dhet* females bred to *Src-1/2 dhet* males and discovered that SP-A expression was significantly decreased in the lungs and amniotic fluid

of all doubly deficient fetuses, compared to WT. By contrast, SP-A in lungs and amniotic fluid of fetuses that were singly deficient in *Src-1* or *Src-2* were no different from WT (Fig. 4D–F). In addition, myometrial NF- κ B activation, expression of *Oxtr*, *Cx43*, *PGF₂ α synthase/Akr1b3* were significantly reduced in myometrial tissues of *Src-1/2 dhet* females bred to *Src-1/2 dhet* males, and from WT females bred to *Src-1-KO/Src-2-het* males, whereas, expression of the prostaglandin degrading enzyme, prostaglandin dehydrogenase (PGDH) was significantly increased. This also was associated with a significant decrease in levels of the contractile prostaglandin, PGF₂ α , in myometrium of *Src-1/2-dhet* or WT females crossed with *Src-1/2*-doubly deficient males [33]. Since increased uterine PGF₂ α is known to circulate to the ovary and promote luteolysis resulting in a decline in P₄ production in most sub-primate species [96], we analyzed ovarian tissues from these genetic crosses for expression of steroidogenic acute regulatory protein (StAR), a key regulator of cholesterol metabolism to progestins [97]. We observed that StAR protein and mRNA failed to decline in corpora lutea of *Src-1/2-dhet* females bred to genetically-like males and from WT females bred to *Src-1/2* double-deficient males at term; consequently circulating P₄ remained elevated [33].

Clearly, the parturition delay in mice carrying *Src-1/Src-2* doubly deficient fetuses (~38 h) was markedly greater than that observed in *SP-A-KO* and in *SP-A/SP-D-dKO* mice (~12 h) (Fig. 2). This suggests that other fetal factors are impacted by *Src-1/2* double deficiency. The finding that *Src-1/Src-2 dKO* mice die at birth of respiratory distress [95] indicated that surfactant lipid components also were affected. In this regard, we found that levels of the highly surface-active glycerophospholipid, DPPC, were significantly decreased in amniotic fluid from *Src-1/Src-2* double-deficient fetuses as compared to WT [33].

As mentioned, PAF, an inflammatory glycerophospholipid produced by the developing fetal lung and secreted into amniotic fluid was previously suggested to contribute to the initiation of term and preterm labor [34,56,98–100]. PAF levels were increased in amniotic fluid of women in preterm labor [98,101]. Studies with PAF receptor KO mice suggested that PAF acts on polymorphonuclear leukocytes to stimulate their activation [102]. Moreover, PAF enhanced cytokine production and migration of PMNs into the cervix at time of parturition and directly stimulated contraction of myometrial strips [103–106]. Accordingly, we found that PAF levels failed to increase toward term and were significantly reduced in fetal lungs and amniotic fluid of *SRC-1/2* double-deficient mice, compared to WT or *Src-1* or *Src-2* singly-deficient fetuses at 18.5 dpc (Fig. 5A, B).

In considering the glycerophospholipid metabolizing enzymes that might coordinately regulate both DPPC and PAF synthesis in fetal lung, we discovered that expression of lysophosphatidylcholine acyltransferase 1 (*Lpcat1*), a key enzyme in the remodeling/acylation of both DPPC and PAF [107–109], was decreased in lungs of *Src-1/2 dKO* fetuses compared to WT. *Lpcat1* previously was reported to be specifically expressed in mouse lung type II cells, upregulated in fetal lung toward term, and stimulated by glucocorticoids [108]. We observed that the developmental upregulation of *Lpcat1* was prevented in lungs of *Src-1/2* double-deficient fetuses (Fig. 5C). Moreover, a significant decrease in *Lpcat1* mRNA was evident only in lungs of fetuses that were doubly-deficient for *Src-1/2*, compared to WT (Fig. 5D). The finding that mice homozygous for a hypomorphic allele of

Lpcat1 manifested respiratory distress at birth, with an associated deficiency in surfactant DPPC [107], underscores the importance of LPCAT1 in fetal lung surfactant synthesis.

In studies using cultured human fetal lung type II cells, we observed that the synthetic glucocorticoid dexamethasone induced LPCAT1 expression in a GR/SRC-1/SRC-2-dependent manner. Glucocorticoid upregulation of *LPCAT1* promoter activity was mediated by two consensus GRE repeats that bound endogenous GR, SRC-1 and SRC-2 in a cooperative manner [33]. Previous findings that *Src-1-KO* and *Src-2-KO* mice manifested hyposensitivity to glucocorticoids [110] further suggest that compromised action of glucocorticoids in *Src-1/Src-2*-deficient fetal mice may contribute to decreased *LPCAT1* expression and reduced production of DPPC and PAF.

Because both PAF and SP-A were decreased in fetal lungs and amniotic fluid of *Src-1/-2* double-deficient fetuses, we determined whether administration of either of these factors could prevent the parturition defect. Importantly, intraamniotic injection of either PAF or SP-A at 17.5 dpc corrected the parturition delay in *Src-1/-2* deficient mice and restored increased NF- κ B activation, Cx43 and Oxt expression in the maternal myometrium, StAR expression in the ovary and circulating P₄ levels to WT values at 18.5 dpc [33].

These collective findings suggest that SP-A and PAF produced by the maturing fetal lung comprise important signals for the initiation of parturition (Fig. 6). SRC-1 and SRC-2, serve critical roles through transcriptional upregulation of genes encoding SP-A and LPCAT1, a key regulatory enzyme in the synthesis of surfactant DPPC and PAF, a potent proinflammatory glycerophospholipid. The surfactant components SP-A and PAF activate immune cells, which infiltrate the maternal uterus to cause an inflammatory response leading to a decline in PR function. This promotes the increased expression of myometrial contractile genes and enhanced production of PGF₂ α , which can circulate to the ovary and cause luteolysis, resulting in decreased P₄, which further enhances the myometrial inflammatory response, culminating in the initiation of labor. Thus, the fetus contributes to the timing of its birth by signaling the mother when its lungs can produce sufficient surfactant DPPC to transition from an aqueous to an aerobic environment.

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Highlights

- Progesterone acting via PR maintains myometrial quiescence during pregnancy.
- This occurs via inhibition of inflammatory and contractile genes.
- Myometrial contractility is induced by increased inflammatory signaling.
- The fetal lung secretes signals that contribute to the initiation of labor at term.
- Signals include surfactant protein-A (SP-A) and platelet-activating factor (PAF).

Fig. 1. Mechanisms for P₄/PR maintenance of myometrial quiescence throughout most of pregnancy (**left panel**) and for the initiation of term and preterm parturition (**right panel**). During pregnancy, increased P₄ acting through PR can maintain myometrial quiescence via several mechanisms, including: interaction with NF- κ B p65 and recruitment of co-repressors to block p65 transcriptional activation; transcriptional induction of the NF- κ B inhibitor, I κ B α , and/or the MAPK inhibitor, MKP-1/DUSP1, and; transcriptional induction of ZEB1, which suppresses transcription of myometrial contractile genes. The initiation of labor at term (by increased secretion of surfactant components from the fetal lung, increased uterine stretch and increased circulating E₂) and preterm (by infection with chorioamnionitis) is accompanied by activation of macrophages that may be of fetal origin, their migration to the maternal uterus and release of proinflammatory cytokines. This results in an increase in myometrial NF- κ B and ER α transcriptional activity and upregulation of contractile and proinflammatory genes, as well as a decline in PR function, which further accelerates the inflammatory process leading to labor.

Fig. 2. Deficiency of surfactant proteins SP-A and SP-D in mice causes a delay and dysregulation of the timing of parturition (**A, B**), as well as impaired upregulation of contractile genes (Cx43 and Oxtr) in the maternal myometrium at term (**C, D**). Reproduced from [35].

Fig. 3.

A 300-bp region upstream of the *SP-A* gene assembles an enhanceosome that mediates developmental, type II cell-specific and hormonal regulation of expression in fetal lung. Studies in transgenic mice and transfected type II cells indicate that a 300-bp region upstream of the *SP-A* gene mediates its developmental, tissue-specific and hormonal regulation of expression. Endogenous and exogenous signals (cAMP, IL-1) induce phosphorylation, activation and DNA-binding of TTF-1, NF- κ B and ERR α . Phosphorylated TTF-1, NF- κ B p65 and ERR α bound to response elements recruit SRC-1 and SRC-2 coactivators and the histone acetylase, CBP/p300. The opening of chromatin structure permits binding of transcription factor IID (TFIID) and stable assembly of components of the RNA polymerase II preinitiation complex (RNAPII PIC).

Fig. 4.

The marked parturition delay in *Src-1/-2 dhet* female mice crossed with *Src-1/-2 dhet* males is associated with reduced SP-A expression in lungs of *Src-1/-2* double-deficient fetuses. **(A)** Expected fetal genotypes from *Src-1/-2dhet* crosses and gestation length. **(B)** Gestation length is plotted according to genotypes of males and females. **(C)** Gestation length of *SRC-1/-2dhet* females bred to *Src-1/-2 dhet* males is positively correlated with proportion of *Src-1/Src-2* double-deficient pups. **(D)** SP-A mRNA levels according to genotype at 18.5 dpc from matings of *SRC-1/-2dhet* females $\times$ *SRC-1/-2dhet* males. **(E)** Levels of SP-A protein in 18.5 dpc fetal lungs from *Src-1/-2dhet* females bred to *Src-1/-2dhet* males. Upper panel: representative immunoblot of SP-A protein according to genotype. Lower panel: data from scans of SP-A immunoblots normalized to β -actin according to fetal genotype. **(F)** SP-A protein levels in AF surrounding WT and *Src-1/Src-2* deficient fetuses. Data are the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (ANOVA). Reproduced from [33]. Please see original source for details regarding numbers of mice studied.

Fig. 5.

PAF secretion and *Lpcat1* expression are reduced in lungs of *SRC-1/-2* double-deficient fetuses. **(A)** PAF levels increase in amniotic fluid surrounding WT fetuses during late gestation. **(B)** PAF levels fail to increase in amniotic fluid of *Src-1/-2* double-deficient fetuses at 18.5 dpc compared to *Src-1* or *Src-2* single KO and WT. **(C)** *Lpcat1* mRNA levels in lungs of *Src-1/-2 dhet* fetuses are significantly decreased compared to WT at 18.5 and 19.5 dpc. **(D)** *Lpcat1* mRNA levels are significantly decreased in lungs of *Src-1/-2* double-deficient fetuses compared to WT and to lungs of fetuses with deficiency in either *Src-1* or *Src-2*. Data are mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; # $P < 0.05$, ## $P < 0.01$ compared to WT at same dpc (ANOVA). Adapted from [33]. Please see original source for details regarding numbers of mice studied.

Fig. 6.

Model: SRC-1/2 regulation of *SP-A* and *LPCAT1* in fetal lung promote a signaling cascade leading to the initiation of labor. During the last trimester of gestation, enhanced binding of TTF-1 and NF-κB to the TTF-1-binding element (TBE) upstream of the *SP-A* gene and of glucocorticoid receptor (GR) to glucocorticoid-response elements (GREs) upstream of the *LPCAT1* gene recruit SRC-1 and SRC-2, resulting in activation of *SP-A* and *LPCAT1* expression. Increased SP-A and PAF secretion into amniotic fluid activates fetal macrophages which migrate to the maternal myometrium, resulting in an inflammatory response with activation of NF-κB and increased transcription of the contraction-associated protein (CAP) genes, *OXTR*, *CX43* and *PDGF α synthase*. Increased $PGF_{2\alpha}$ is transported to the ovary where it promotes luteolysis and decreased StAR expression, resulting in a decline in circulating P_4 , which further enhances the myometrial inflammatory response, leading to labor. Reproduced from [33].