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Ronald McCarthy
Washington University School of Medicine in St. Louis

Carmel Martin-Fairey
Washington University School of Medicine in St. Louis

Dorothy K. Sojka
Washington University School of Medicine in St. Louis

Erik D. Herzog
Washington University in St. Louis

Emily S. Jungheim
Washington University School of Medicine in St. Louis

See next page for additional authors

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Review
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Review

Mouse models of preterm birth: suggested assessment and reporting guidelines†

Ronald McCarthy[1], Carmel Martin-Fairey[1], Dorothy K. Sojka[2], Erik D. Herzog[3], Emily S. Jungheim[4], Molly J. Stout[4], Justin C. Fay[5], Mala Mahendroo[6], Jeff Reese[7], Jennifer L. Herington[7], Erin J. Plosa[7], Elaine L. Shelton[7] and Sarah K. England[1,*]

[1]Center for Reproductive Health Sciences, Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, Missouri, USA; [2]Rheumatology Division, Washington University School of Medicine, St. Louis, Missouri, USA; [3]Department of Biology, Washington University in St. Louis, St. Louis, Missouri, USA; [4]Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, Missouri, USA; [5]Department of Biology, University of Rochester, Rochester, New York, USA; [6]Department of Obstetrics and Gynecology University of Texas Southwestern Medical Center, Dallas, Texas, USA and [7]Department of Pediatrics, Vanderbilt University Medical Center, Nashville, Tennessee, USA

*__Correspondence:__ Department of Obstetrics and Gynecology, Washington University in St. Louis School of Medicine, 425 South Euclid Avenue, Campus Box 8064, St. Louis, MO 63110. Tel: +(314) 286–1798; Fax: +(314) 747–4150; E-mail: englands@wustl.edu

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Abstract

Preterm birth affects approximately 1 out of every 10 births in the United States, leading to high rates of mortality and long-term negative health consequences. To investigate the mechanisms leading to preterm birth so as to develop prevention strategies, researchers have developed numerous mouse models of preterm birth. However, the lack of standard definitions for preterm birth in mice limits our field's ability to compare models and make inferences about preterm birth in humans. In this review, we discuss numerous mouse preterm birth models, propose guidelines for experiments and reporting, and suggest markers that can be used to assess whether pups are premature or mature. We argue that adoption of these recommendations will enhance the utility of mice as models for preterm birth.

Summary Sentence

To improve reporting of mouse models of preterm birth, a set of universal guidelines and simple assays of developmental markers are proposed to distinguish between mature and premature pups.

Key words: preterm birth, mouse models, pregnancy, gestation, parturition.

Introduction

Every year, approximately 15 million babies (10% of all births worldwide) are born preterm, defined as delivery before 37 weeks of gestation [1–3]. Preterm birth is the leading cause of infant mortality [4], and those born prematurely have increased lifelong risks of adverse health outcomes including cognitive impairment, cardiovascular disease, and chronic pulmonary disease [5]. In approximately

one third of preterm births, labor is induced or cesarean section is performed because of maternal or fetal complications such as preeclampsia, intrauterine growth restriction, or fetal distress. However, the majority of preterm births are spontaneous, brought about by preterm labor (uterine contractions leading to cervical change and delivery) or preterm premature rupture of fetal membranes (PPROM) [6, 7]. Unfortunately, the multiple causes of preterm labor have not been fully determined, and we have limited ability to predict or prevent preterm birth [8–10].

To address these limitations, researchers have developed various mouse models of preterm birth [11]. Mice are useful for studying the timing of birth because they have a short gestation, can easily be genetically manipulated, are inexpensive, and can be studied in large numbers [12]. Additionally, key components of the labor and delivery process are conserved between mice and humans. For example, the expression levels of several proteins that activate uterine contractions including prostaglandins, oxytocin, and connexin-43 are elevated at term in both species [11, 13–15]. Finally, parturition timing is somewhat variable in both humans, in whom 90% of births occur between 37 and 42 weeks of gestation, and mice, in which gestation lengths vary between strains from 19 to 21 days [16].

Despite these similarities, three key physiological differences between human and mouse pregnancy are worth mentioning. First, whereas women typically have singleton pregnancies [17] within a single uterine cavity, mice commonly have 4 to 10 pups per litter (depending on strain) [16] within two uterine horns. This can complicate comparisons between species because women carrying multiple pregnancies are at increased risk for preterm birth [18]. Second, although progesterone is required to maintain pregnancy and is initially produced by the corpus luteum in both humans and mice, its production thereafter is regulated differently in the two species. In humans, the placenta takes over progesterone production after gestational week 7 or 8, and progesterone levels rise fairly continuously until the end of pregnancy [19, 20]. At the end of human pregnancy, a shift in progesterone receptor isoform expression and local increases in progesterone metabolism [21, 22] result in a functional withdrawal of the "pregnancy-maintaining" hormone. In mice, progesterone synthesis and levels decrease while local progesterone metabolism [23] increases dramatically in the last two days of pregnancy, facilitating the onset of parturition. Third, although estrogens inhibit uterine quiescence and promote cervical ripening after progesterone action decreases (by promoting production of uterotonins and contraction-associated proteins, including: connexin-43, oxytocin receptor, cyclooxygenase 2, and prostaglandin F receptor) in both humans and mice, estrogen is regulated differently in the two species. In humans, circulating estrogens, derived from the ovary and placenta, are high throughout gestation. In mice, estrogens are synthesized by the ovary at low levels in the first half of pregnancy and then markedly increase in the latter half of gestation.

As we detail in this review, "preterm birth" is loosely defined for mice, and our field lacks clear, accepted guidelines for conducting experiments and reporting results. Thus, we cannot easily compare mouse studies and use their results to improve our ability to predict and prevent preterm birth in women. This review seeks to fill this gap by describing mouse preterm birth models published in the literature and the ways in which they aim to represent the human condition. Additionally, we discuss experimental differences leading to inconsistent definitions of preterm birth in mice and propose criteria to provide more uniform assessment of mouse models. Finally, we suggest that investigators should assess and report the developmental status (mature or premature) of offspring. Toward this end, we describe promising fetal developmental markers that can be used to distinguish between mature and premature pups.

Mouse models of preterm birth

Here, we review the major classes of mouse preterm birth models, categorized by pathologic state. Representative examples are provided in Table 1.

Infection

Multiple studies have implicated activation of inflammatory pathways in the process of normal uncomplicated labor (as reviewed in [24]). However, early initiation of the inflammatory pathway by infection contributes to between 25% and 40% of all human preterm births [25–27]. Infections can develop in two major ways. Chorioamnionitis or intrauterine infection can arise systemically, or commensal bacteria can ascend from the female genital tract. Several mouse models of infection-induced preterm birth have been developed [28, 29], most commonly using *Escherichia coli* or the toxic component on the surface of gram-negative bacteria, lipopolysaccharide (LPS). Other bacteria associated with increased risk of preterm birth in humans, and therefore studied in mice, include *Ureaplasma*, *Chlamydia trachomatis*, Group B streptococcus (GBS; *Streptococcus agalactiae*), and *Porphyromonas gingivalis*. To mimic local infection, investigators have performed intrauterine, intraamniotic, or intraperitoneal injections with live or heat-killed bacteria. To simulate systemic infection, they administer injections intraperitoneally, and to mimic ascending infections, they inject bacteria vaginally or via the intrauterine route [30–32].

Mice injected with live or heat-killed *E. coli* or LPS on 14.5–15.5 days post coitus (dpc) delivered within 7–48 h, depending on the route of administration and LPS serotype used. Specific *E. coli*-derived LPS serotypes differentially activated proinflammatory responses, caused mice to deliver at different times postinjection, and were associated with varying levels of offspring survival [33]. Both *E. coli* and LPS stimulate the Toll-like receptor (TLR)-4 in the uterus and activate an inflammatory cascade [34]. Further investigation of localized intrauterine inflammation revealed that the TLR-4 pathway stimulated platelet activating factor, an important mediator of the signal transduction pathway that led to inflammatory-induced preterm delivery [35]. In addition, LPS administered vaginally has been shown to work through the complement receptor C5a, affecting cervical remodeling by increasing metalloproteinase-9 activity and collagen degradation [36].

Intrauterine injections of lipoteichoic acid (an anionic polymer on the surface of gram-positive bacteria), peptidoglycan (also a major component on the surface of gram-positive bacteria), or polyinosinic acid (an analog of viral double-stranded RNA) caused mice to deliver preterm by mechanisms similar to that of LPS, albeit through different TLRs [37, 38]. Specifically, administration of the bacterial peptidoglycan-derived peptide γ-D-glutamyl-meso-diaminopimelic acid, an agonist of the pattern recognition receptor Nod-1, caused mouse preterm birth via maternal-fetal inflammation [39].

In humans, GBS is the leading cause of perinatal infection, and maternal GBS infection increases the risk for preterm birth [40, 41]. To model this etiology, Hirsch and colleagues injected pregnant mice (intraperitoneal or intrauterine) with 10^9 heat-killed GBS on 14.5 dpc [42]. In this model, approximately 86% of pregnant mice delivered within 18 h and showed signs of placental and membrane

Table 1. Published preterm birth mouse models.

Type of model	Targeted mutation	Mouse strain	Expected gestational length (days)[a]	Breeding method	Defined start of pregnancy[b]	Stimulant			Preterm delivery definition	Term delivery definition in paper	Pup survival	Reference
						Treatment/ experimental manipulation	Route[c] and concentration	Day of injection				
Infection												
	N/A	CD-1	19–20	ND	Vaginal plug; dpc not defined	*E. coli* 2 – 10×10^5	IU/IP	14.5 dpc	Within 48 h	ND	None	[31]
	N/A	C3H/HeN x C3H/HeN C3H/HeN x B6D2F1 BALB/c x B6D2F1	19–20	ND	Vaginal plug and spermatozoa in vaginal smear at 0.0 dpc	LPS serotype 055:B5	IP - 50 or 100 μg/kg (one injection) Or 50 μg/kg (two injections)	12, 15, or 17 dpc	<19 dpc	19–20 dpc	None	[32]
	N/A	CD-1	ND	ND	Vaginal plug at 0.0 dpc	LPS serotypes 0111:B4, 055:B5, 0127:B8*, and 0128:B12	IU 20 μg in 25 μl	16 dpc	36 h postinjection	60 ± 15 h postinjection	LPS 0111:B4 none 055:B5 80% 0127:B8 95% 0128:B12 100%	[33]
	TLR-4 mutant	CD-1 C3H/HeJ	19–20	Timed pregnant (supplier)	ND	LPS serotype L2280 (CD-1) L2880 and L4525 for TLR-4 mutant	IU 250 μg/mouse (Sigma)	15 dpc	At least 1 pup born < 48 h after LPS admin	19–20 dpc	None	[35]
	C5aR−/−	129S4/SvJae		ND	Vaginal plug at 0 dpc	No treatment, LPS serotype 055:B5 or RU486	250 μg LPS intravaginally SC 150 μg RU486 dissolved in DMSO	15 dpc	Within 48 h postinjection	20–21 dpc	ND	[36]
	N/A	CD-1	19–20	ND	Vaginal plug, dpc not defined	TLR-2 ligand lipoteichoic acid, peptidoglycan, or TLR-3 ligand polyi-nosinic:cytidylic acid	IU and IP	14.5 or 15.5 dpc	At least 1 pup born or in lower vagina <48 h after surgery	19–20 dpc	None	[37]
	N/A	C3H/HeNCrj X Crj: B6D2F1	19–20	ND	Vaginal plug and spermatozoa in the vaginal smear at 0.0 dpc	Lipoteichoic acid	IP 12.5–75 mg/kg single dose or repeated doses at 3h intervals	15 dpc 17 dpc	<19.0 dpc	19–20 dpc	None with doses given on 15 dpc 100% viable with doses given on 17 dpc	[38]
	N/A	C57BL/6	19.5 ± 0.5	ND	ND	iE-DAP	IP 500, 750, or 1000 μg in 200 μl PBS	14.5 dpc	Delivery within 24 h postinjection	ND	ND	[39]
	N/A	CD-1	ND	Timed pregnant (supplier)	ND	Heat-killed GBS	IP or IU 10^9 in 100 μl	14.5 dpc	Within 48 h postinjection	ND	ND	[42]

Table 1. Continued

Type of model	Targeted mutation	Mouse strain	Expected gestational length (days)[a]	Breeding method	Defined start of pregnancy[b]	Stimulant			Preterm delivery definition	Term delivery definition in paper	Pup survival	Reference
						Treatment/ experimental manipulation	Route[c] and concentration	Day of injection				
	N/A	C3H/HeN	19–20	ND	Vaginal plug at 0.0 dpc	Ureaplasmal outer membrane lipoprotein	IU 15 μg	14.0 dpc	Within 48 h	ND	ND	[45]
	N/A	BALB/c (*H-2*^{d})	19–20	Overnight	Vaginal plug at 0 dpc	*Chlamydia trachomatis* mouse pneumonitis biovar (strain Nigg II)	10^{1} to 10^{7} inclusion-forming units in 20 μl of 0.2 M sucrose–20 mM sodium phosphate (pH 7.2)–5 mM glutamic acid	5 dpc	ND	19.3	Maternal cannibalism precluded evaluation	[47]
	N/A	C3H/HeJ	20	Timed-pregnant (supplier)	ND	*E. coli* Dr^{+} IH11128 and Dr14	Urethral catheterization into urinary bladder	7 dpc	On or before 18 dpc	ND	53.6%	[50]
	N/A	C57Bl/6J	20.45	ND	Vaginal plug at 0.0 dpc	W83 strain of *P. gingivalis*	10^{8} CFU	6 weeks before mating	17–18.25 dpc	Delivery	ND	[55]
	Faah–/–; Cnr–/–	ND	~19.8	ND	Vaginal plug at 1.0 dpc	LPS 0111:B4	IP 25 μg	16.0 dpc	Before 19 dpc	~19.8 dpc	None	[139]
	N/A	Kunming (derived from Swiss Webster)	ND	Overnight	Vaginal plug at 0.0 dpc	LPS serotype 0127	IP 150 μg/kg	15.5 dpc	15.5–17 dpc	18.0–20.0 dpc	47%[d]	[140]
	N/A	CD-1	19–20	Overnight	Vaginal plug at 0.0 dpc	LPS serotype 0111	IU 10 μg	16.0 dpc	12.7 ± 7 h	ND	14.6 ± 31% viable, but included deliveries 25–37 h after LPS	[141]
	N/A	C57BL/6	19.5 ± 0.5	Overnight	Vaginal plug at 0.5 dpc	LPS	IP 0.5 μg	16.5 dpc	Before 18.0 dpc	ND	2.3 fetuses per dam, may include term deliveries	[142]
	N/A	C57BL/6	19.5 ± 0.5	ND	Vaginal plug at 0.5 dpc	LPS serotype 0111:B4	IA 100 ng/sac	16.5 dpc	Before 18.0 dpc	ND	ND	[143]
	Uterine-specific p53 knockout	FVB/129	ND C57BL/6-129	Overnight	Vaginal plug at 1.0 dpc	LPS	IP 10 μg/ml	16.0 dpc	Before 19 dpc	ND	28% w/o LPS; 0% with LPS	[84]
Inflammation												
	N/A	C3H/Hel	20–21	Timed pregnant (supplier)	ND	Interleukin-1	SC	15–17 dpc	Within 24 h	20–22 dpc	ND	[61]
	N/A	C57BL/6	19.5 ± 0.5	Overnight	Vaginal plug at 0.5 dpc	High-mobility group box-1	IA 9 ng	14.5 dpc	17.35 dpc	19.5 ± 0.5 dpc	~85.4% viability; 60.9 ± 11.7% pup death by 1 week of age	[64]

Table 1. Continued

Type of model	Targeted mutation	Mouse strain	Expected gestational length (days)[a]	Breeding method	Defined start of pregnancy[b]	Stimulant			Preterm delivery definition	Term delivery definition in paper	Pup survival	Reference
						Treatment/ experimental manipulation	Route[c] and concentration	Day of injection				
Cesarean												
	N/A	CD-1	20	Overnight	Vaginal plug at 1.0 dpc	Cesarean	NA	NA	On 18 or 19 dpc	20 dpc	0% (18 dpc) 87.8% (19 dpc)	[65]
	N/A	CD-1	19–20	Timed pregnant (supplier)	ND	Cesarean	NA	NA	1 or 2 days before term	Delivery	100%	[144]
PPROM												
	N/A	C57BL/6	19.5 ± 0.5	Pair mated (5 h)	ND	Fetal fibronectin	Between fetal membranes and uterine lining (100–200 μg/ml)	17.0 dpc	<18.5 dpc	19 dpc	20%	[70]
	Biglycan and Decorin double knockout	C3H	19.5	Overnight	Vaginal plug at 0.0 dpc	NA	NA	NA	Before 18.0 dpc	19.5 dpc	0%	[67]
Early progesterone withdrawal												
	N/A	C3H/HeN	19–20	Overnight	Vaginal plug at 1.0 dpc	Mifepristone (RU486)	SC 50–250 μg	12–14 dpc	Within 18 h	19–20 dpc	100%	[75]
Prostaglandins												
	N/A	C3H/HeN	19–20	Timed Pregnant (supplier)	ND	Prostaglandin $F_{2\alpha}$	IP 20 μg	16 dpc	Within 24 h	<18 dpc	ND	[82]
	15-hydroxy-prostaglandin dehydroge-nase hypomorph	C57BL/6-129/SvJ	~19.3	ND	Vaginal plug, dpc not defined	NA	NA	NA	Shorter gestation than control	~19.3 dpc	Equivalent to term	[80]
Uterine quiescence												
	N/A	CD-1	19	Timed pregnant (supplier)	ND	Tunicamycin	IP 0–1 mg/kg	15 dpc	18–32 h postinjection	19 dpc	Nonviable neonates at 16 and 17 dpc	[89]
Endocannabinoid signaling												
	CB1 knockout	C57BL/6J/129	~20.1	Overnight	Vaginal plug at 1.0 dpc	NA	NA	NA	Before ~19.5 dpc	~20.1 dpc	Yes	[99]
Hyperhomocysteinemia												
	CBS knockout	C57BL/6J	20.0 ± 0.2	Overnight	Vaginal plug at 0.5 dpc	NA	NA	NA	At 16.6 ± 0.1 dpc	20.0 ± 0.2 dpc	Yes	[102]

Table 1. Continued

Type of model	Targeted mutation	Mouse strain	Expected gestational length (days)[a]	Breeding method	Defined start of pregnancy[b]	Stimulant			Preterm delivery definition	Term delivery definition in paper	Pup survival	Reference
						Treatment/ experimental manipulation	Route[c] and concentration	Day of injection				
Environmental effects												
	N/A	C57BL/6	19.5 ± 0.5	Overnight	Vaginal plug at 0.5 dpc	2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin)	Mother was gavaged 10 μg/kg; no exposure as adult	In utero at 15.5 dpc, none as adult	24 h before term	20 dpc	Pups born preterm appeared viable at birth, died within 24 h Pups born at term survived	[105]
	N/A	BL6C3F1	20.3 ± 0.2	Two days	First day of pairing defined as 0.0 dpc	Cigarette smoke diluted 90%	Inhalation	2–18 dpc	At 19.6 ± 0.2 dpc	20.3 ± 0.2 dpc	Yes	[145]
Other												
	N/A	CD-1	19–20	Overnight	Vaginal plug at 0 dpc	L-arginine analog N^G-nitro-L-arginine methyl ester (L-NAME)	SC 0 (vehicle), 40, 70, or 100 mg L-NAME/kg in 10 ml/kg body weight	15.5 dpc and 16 dpc	Before 18 dpc	18–19.5 dpc	Maternal cannibalism precluded evaluation	[107]
	N/A	CD-1	19–20	Overnight	Vaginal plug at 0 dpc	Methylene Blue	SC 5, 30, 50, 60 or 85 mg/kg in 5 ml/kgb	15.5 dpc and 16 dpc	Before 18 dpc	18–19.5	Appeared viable but maternal cannibalism precluded evaluation	[108]
	N/A	ICR (CD-1)	19–20	Overnight	Vaginal plug at 0.5 dpc	Surfactant protein (SP)-A	IA 3 μg in 50 μl per sac	15 dpc	ND	19 dpc	ND	[109]
	N/A	BALB/C	20.2 ± 0.1	Overnight	Vaginal plug at 0 dpc	Neuromedin B	IP 30, 90, or 150 μg/kg of NMB	18 dpc and 19 dpc at 1400 and 1800 h	ND	ND	ND	[110]
	N/A	C57Bl/6J	19.5 ± 0.5	ND	Vaginal plug at 1 dpc	Alcohol	Intra-gastric 6 g/kg	17 dpc or 18 dpc	18.9 ± 0.1 dpc 19.5 ± 0.2 dpc	20.1 ± 0.1	ND	[111]

[a] As discussed by author or determined by [16]
[b] dpc, days postcoital
[c] IA, intraamniotic; IP, intraperitoneal; IU, intruterine; SC, subcutaneous
[d] includes term deliveries
NA, not applicable
ND, not discussed
LPS, Lipopolysaccharide

apoptosis. In another model, 75% of mice that were vaginally colonized with GBS on 16 dpc delivered preterm. However, 0% of mothers that were vaccinated with GBS before mating delivered preterm, and their pups had higher survival rates and lower levels of neonatal GBS infection than those from unvaccinated mothers [42].

Another bacterial genus that commonly infects the uterus and causes preterm birth in humans is *Ureaplasma*, a member of the *Mycoplasmataceae* family [43]. Additionally, *Ureaplasma* is the most frequently isolated bacterial pathogen in cases of chorioamnionitis [44]. One group reported that injecting a diacylated lipopeptide derived from *Ureaplasma parvum* into the uterus of pregnant mice on 15 dpc resulted in preterm delivery, although the day of delivery was not defined. This work revealed that *Ureaplasma* likely induced preterm birth by binding to TLR-2 and activating the NF-κB inflammatory cascade [45].

The pathogen *Chlamydia trachomatis* is the most prevalent sexually transmitted bacteria and is associated with preterm delivery [46]. Mice infected with 10^5–10^7 inclusion-forming units (IFU) of *C. trachomatis* delivered on 15.8–16.4 dpc, whereas those infected with 10^1–10^4 IFU delivered on 19.5–19.6 dpc, and those that received vehicle or no treatment delivered had a mean gestation length of 19.3 dpc [47]. Acute to severe inflammation and *C. trachomatis* inclusions were noted in the maternal uterine wall, endometrium, and fetal membranes but were absent in the amnion and fetal organs.

Urinary tract infections, most commonly caused by *E. coli* [48], have long been known to be associated with increased risk for preterm birth in humans [49]. To model this etiology, mice were infected with *E. coli* via urethral catheterization into the urinary bladder on 7 dpc. Nearly 90% of those infected with bacteria expressing the Dr adhesin delivered between 11 and 18 dpc [50], whereas only 10% of those infected with *E. coli* not expressing this Dr adhesin delivered preterm. *Escherichia coli* expressing Dr adhesin were able to colonize the kidneys and spleen of the mothers and transfer through the placenta to the fetuses, causing reduced fetal weight and poor organ development.

Maternal infections distant from the uterus have also been implicated in preterm birth. For example, some data suggest that maternal periodontal disease increases the risk of preterm birth by sevenfold [51]. Periodontal pathogens are capable of entering the bloodstream and spreading throughout the body and have been detected in the amniotic fluid [52, 53]. Moreover, *P. gingivalis* antigens have been detected in the placentas of women with chorioamnionitis [54]. In a mouse model of periodontal infection, *P. gingivalis* was injected into the first molar chambers of female mice 6 weeks before mating. Infected mice delivered 2 days earlier and had higher circulating levels of the proinflammatory cytokines tumor necrosis factor-alpha, interleukin (IL)-17, IL-6, and IL-1B than noninfected mice. Additionally, the bacteria were found in the placentas, and the mothers showed features of PPROM and placental abruption [55].

Inflammation

Inflammation in the absence of overt infection is a common etiology of preterm birth. In fact, sterile intra-amniotic inflammation is associated with ~26% of all preterm deliveries and is more common in early than in late preterm deliveries [56, 57]. An important contributor in sterile intra-amniotic inflammation is the cytokine IL-1, which is expressed in the human decidua, promotes prostaglandin production, and is detected at high levels in the uterus of women who deliver preterm [58]. IL-1 stimulates preterm birth in rabbits and nonhuman primates [59, 60]. To model this in mice, researchers injected pregnant mice with IL-1 three times between 15 and 17 dpc, leading to delivery within 24 h [61]. Other important players in sterile intra-amniotic inflammation are danger signals, such as damage-associated molecular pattern molecules and alarmins, which, upon stimulation by cellular stress and necrosis, activate the innate immune system [62]. One such alarmin, high mobility group box 1 (HMGB1), is increased in women with intra-amniotic inflammation [63]. To model this etiology of preterm birth, researchers injected HMGB1 into the amniotic sacs of fetuses on 14.5 dpc; 57% of the injected mice delivered by 17.5 dpc, whereas all of the controls delivered at full term (19.5 dpc) [64].

Cesarean section

To benefit maternal or fetal health, preterm cesarean delivery may be performed after either medically indicated or spontaneous preterm labor. To investigate the effect of cesarean delivery on fetal development and mortality, researchers surgically removed mouse fetuses before the normal delivery date and found that pups of the strain CD-1 could only be resuscitated when delivered on 19 or 20 dpc (20 dpc was the normal delivery time for this strain in their colony) [65], indicating that mice cannot survive outside the uterus if birth occurs more than 1 day preterm.

Preterm premature rupture of membranes

In women, PPROM is a significant pregnancy complication. In this condition, the chorioamniotic membrane surrounding the fetus ruptures before 37 weeks of pregnancy, breaching the barrier between the extrauterine and intrauterine environment, allowing the amniotic fluid to leak out, and increasing the risk for infection and preterm birth. PPROM can be caused by infection, overdistension of the uterus and amniotic sac (such as in multiple pregnancies), trauma, or genetic disorders. For example, women with Ehlers-Danlos syndrome carry mutations in the genes encoding proteins involved in assembly of collagen and elastic fibers and are at increased risk of PPROM, cervical insufficiency, uterine rupture, and delivering an intrauterine growth-restricted fetus [66]. C3H mice with targeted mutations in genes encoding specific proteoglycans, such as biglycan and decorin, displayed an Ehlers-Danlos-like phenotype that included reduced litter size and intrauterine growth restriction. Additionally, despite lacking an inflammatory response, these mice delivered before 18.0 dpc, with 43% delivering by 17.0 dpc, and none of the fetuses survived [67].

The extracellular matrix glycoprotein fetal fibronectin (fFN) is part of the amniotic membranes, and detection of fFN in cervical and vaginal fluid has been associated with an increased risk for spontaneous preterm birth. However, few patients with a positive fFN result will actually deliver early [10, 68, 69]. In mice, injection of fFN at 17.0 dpc causes preterm delivery within 12–36 h, likely via PPROM induced by activation of matrix metalloproteinases and cyclooxygenase-2 [70]. Likewise, injecting mice with thrombin at 17.0 dpc increased levels of matrix metalloproteinases and cyclooxygenase-2 and caused delivery of non-viable pups within 24 h [70–72].

Early progesterone withdrawal

A successful pregnancy requires uterine smooth muscle quiescence before parturition/labor. During gestation, progesterone is one of the predominant hormones produced by the placenta and is responsible for keeping the myometrium in a quiescent state. Progesterone levels remain high throughout human pregnancy, but women undergo a

"functional" progesterone withdrawal at the end of pregnancy. This reduction in progesterone action is mediated by alterations in the ratio of progesterone receptor A to progesterone receptor B [73], changes in cofactor expression, and/or local metabolism of progesterone, thereby allowing contractions to initiate and the cervix to ripen [74]. Consistent with this idea, the anti-progesterone and anti-glucocorticoid drug Mifepristone, also known as RU486, induces uterine contractions and cervical ripening. To model early progesterone withdrawal, investigators injected mice with RU486 on 12–14 dpc, resulting in delivery within 18 h in ~84% of C3H/HeN mice. Although this study reported that pups were born alive, postnatal pup survival was not discussed [75]. RU486-induced premature cervical ripening in Blk6/129SvEv mice was reported to be similar to term ripening and distinct from LPS-induced premature ripening in terms of gene expression, immune cell populations, and microstructural reorganization of collagen [76, 77]. In other reports, RU486 was suggested to promote cervical remodeling by activating the complement receptor C5a [36].

Prostaglandins

At the end of human pregnancy, levels of the prostaglandins PGE_2 and $PGF_{2\alpha}$ increase, bind to receptors on the uterus, and promote contractions. Clinically, PGE_1 (misoprostol) is used to induce both uterine contractions and cervical ripening (effacement or thinning). As an alternative to giving women exogenous prostaglandins, mechanical compression of the cervix promotes local release of endogenous prostaglandins [78, 79]. To model excess prostaglandin activity in mice, researchers created a C57BL/6-129/SvJ mouse line with a hypomorphic mutation in hydroxyprostaglandin dehydrogenase (PGDH), which hydrolyzes prostaglandins. These mice had elevated levels of PGE_2 and $PGF_{2\alpha}$ and delivered approximately one-half day early [80]. Consistent with these earlier studies, co-administration of PGE2 and a PGDH inhibitor on gestation day 15 induced birth within 12 to 48 h [81]. In another study, C3H/HeN inbred wild-type mice treated with 20 μg of $PGF_{2\alpha}$ on 16 dpc delivered 19.3 h postinjection, whereas vehicle-injected mice delivered 53.5 ± 13.6 h postinjection [82].

Uterine senescence

One hypothesis states that gestation length is governed by the timing of placental and decidual senescence, marked by a reduction in telomere length, and that parturition is initiated when these tissues become "old" [83]. Cellular aging is promoted by signaling through mammalian target of rapamycin complex 1 (mTORC1). The tumor suppressor p53 reduces mTORC1 signaling by activating AMP kinase, thereby inhibiting the aging process. To examine the relationship between uterine senescence and parturition, mice were generated lacking p53 specifically in the uterus. Once pregnant, these mice had decreased AMP kinase activation and increased mTORC1 signaling, resulting in early decidual senescence and increased incidence of preterm delivery (before 19 dpc) in some females [84–86]. Modeling gene–environment interactions, work in this model demonstrated that low-dose LPS injection further increased the incidence of preterm birth [87].

Uterine quiescence

The protease caspase-3 helps maintain quiescence by cleaving contractile proteins during gestation [88]. Condon and colleagues hypothesized that caspase-3 is maintained in an active state during pregnancy by the endoplasmic reticulum (ER) stress response and that its activity is reduced by the unfolded protein response at the end of pregnancy. In support of this model, injecting mice with tunicamycin, which induces excessive ER stress response, on 15 dpc caused early increases in levels of the contractile proteins connexin-43, alpha actin, and gamma actin, and resulted in dose-dependent early onset of labor beginning on 16 dpc. Conversely, co-administration of 4-phenylbutrate, an inhibitor of ER stress, prevented both the increase in contractile proteins and preterm birth [89].

Endocannabinoid signaling pathway

In human pregnancy, levels of the endocannabinoid anandamide are high in early gestation, decrease during mid-gestation, and spike at the onset of labor [90, 91]. Anandamide is thought to affect parturition timing by controlling secretion of corticotropin-releasing hormone (CRH) [92], which regulates the duration of pregnancy and onset of labor [93, 94]. Anandamide and another cannabinoid, 2-arachidonoxylglycerol, signal through the G protein-coupled cannabinoid receptor CB1 [95–98]. Deletion of the CB1 gene caused pregnant mice to go into preterm labor, delivering about one-half day before term. In this model, preterm delivery was thought to occur because of early progesterone withdrawal and increased estrogen production during pregnancy. Additionally, signaling through CB1 may control the CRH/corticosterone endocrine axis, as loss of CB1 caused an early rise in CRH and high levels of corticosterone during pregnancy [99].

Hyperhomocysteinemia

In a case-control study of 651 women, elevated homocysteine in maternal blood (hyperhomocysteinemia) during pregnancy was associated with increased odds of preterm birth [100]. One cause of hyperhomocysteinemia is a mutation in the gene encoding cystathionine B-synthase (CBS), an enzyme in the trans-sulfuration pathway [101]. To test a possible mechanism linking hyperhomocysteinemia to preterm birth, mice were generated carrying a mutation in CBS. $CBS^{-/-}$ pregnant mice had increased blood levels of homocysteine, developed preterm uterine contractions, and delivered significantly early (16.6 dpc vs. 20.2 dpc). Further analysis of this model demonstrated that the contractions were caused by preterm expression of the oxytocin receptor and increased PGE2 synthesis by the enzyme prostaglandin endoperoxide synthase 2 [102].

Environmental effects: cigarette smoking and dioxin exposure

Women who smoke cigarettes during pregnancy are at a higher risk of delivering preterm than nonsmokers [103]. To model this etiology, Ng and Zelikoff exposed mice to cigarette smoke via inhalation between 2 and 18 dpc. The mice exposed to cigarette smoke delivered at 19.6 dpc, whereas unexposed mice delivered at 20.3 dpc; the pups survived in both groups [104]. A major toxic component of cigarettes is 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin), which is also a ubiquitous environmental contaminant, a by-product of the industrialized process, and an endocrine disruptor. Because animals and humans are most sensitive to environmental toxicant exposure during in utero development, Bruner-Tran and Osteen exposed pregnant mice to dioxin and then examined the effects on fertility and preterm birth in the next two generations [105]. They found that 36% of females in the F1 generation delivered preterm as adults. Moreover, 25% of the F2 generation (which were exposed as germ cells of the F1 mice in utero) delivered preterm. Furthermore, when F1 females were exposed to GBS, mouse parvovirus, or LPS, the rates

of preterm birth increased to 83, 86, and 100%, respectively [105, 106]. This model suggests that toxicant exposure acts additively with other risk factors to cause preterm birth.

Other models of PTB

Additional mouse models of preterm birth working through mechanisms of action not discussed above include inhibition of nitric oxide synthesis by treating mice with N^G-nitro-L-arginine methyl ester [107]; inhibition of soluble guanylate cyclase by treating mice with methylene blue [108]; exogenous surfactant protein-A, which initiates the NF-κB-signaling cascade in the uterus [109]; injection of neuromedin B, which acts through its receptor to induce labor onset via the RELA (NF-κB P65)/IL6-mediated pathway [110]; and alcohol-induced preterm birth, which is associated with elevated levels of uterine PGE and $PGF_2\alpha$ and increased expression of contraction-associated proteins and is prevented by pretreatment with the prostaglandin synthesis inhibitor aspirin [14, 111].

Defining preterm birth in mouse models

Given the large number of available mouse preterm birth models, standard definitions would allow researchers to appropriately compare results between studies and make meaningful inferences for human preterm birth. We argue that two related questions should be addressed when considering available and newly developed models: (1) Is the gestational length truly shortened, or does delivery occur within the normal range for the mouse strain? (2) Does the model deliver pups that are developmentally immature at delivery? Below, we draw on representative examples listed in Table 1 to highlight variations in experimental design that complicate our ability to evaluate and compare the current mouse models of preterm birth.

Strain-specific characteristics

Researchers have used several different strains of mice to generate models of preterm birth. The mouse strain is an important consideration given the findings of Murray et al. that gestational lengths can vary by as much as 41.7 h, or a full 1.72 days [16]. For example, the FVB/NJ, C57BL/6J, 129S1/SVIMJ, and A/J strains have average gestational lengths of 450.6, 462.4, 486.3, and 492.3 h, respectively, corresponding to between 18.8 and 20.5 days [16]. Clearly, the strain background has to be considered when determining whether a preterm birth model has shortened gestation.

Approaches for timed matings

Comparing gestational lengths between studies is complicated by variations in breeding protocols. For example, whereas most investigators breed mice overnight, some breed for undefined periods of time, and others obtain timed-mated animals from commercial suppliers. Mating strategies should be standardized given that interlitter variability in fetal body weights is greater when mice are bred continuously than when they are mated for 2 h or overnight [112]. Additionally, our field should standardize the definition of the start of gestation. Investigators record the time at which they see a copulation plug as 0.0, 0.5, or 1.0 dpc. This could result in up to a 24-h difference in timing of gestational length, perhaps leading a delivery to be scored as preterm when it is within the normal variation of delivery time for that mouse strain.

Monitoring the timing of parturition

Multiple methods have been used to monitor the timing of delivery. The onset of parturition is typically defined as the time of delivery of the first pup, which can be precisely determined by using a video camera with infrared lighting to record events in the dark. In contrast, strategies such as checking the dam multiple times during the day and recording the pup number may lead to inaccuracies because newly delivered pups can be hidden in the bedding or may be cannibalized if the dam considers the offspring to be abnormal. Furthermore, mice subjected to frequent disturbances in their cage or a nearby cage may enact a "predator response" defense mechanism that can alter their parturition behavior [113]. If an investigator checks the dam only on the expected morning of delivery, he or she may not realize that offspring were already delivered and thus may report that gestation was longer than it actually was.

Measures of development

Few studies provide comprehensive fetal outcome measurements of their preterm birth models [108]. Typically, studies report survival of offspring born preterm (Table 1), yet few provide details on the pups' developmental status (immature or mature).

Proposed guidelines for defining preterm birth in mouse models

For all studies, investigators should be aware that specific animal facility characteristics, such as diets, bedding, water, animal husbandry, co-bedding with other pregnant dams, environment (conventional vs. barrier systems), and noise pollution, could affect gestation length. Thus, instead of relying on published values, investigators should measure and report term gestational length in wild-type, untreated mice in the same facility in which they will do their experiments. Additionally, we suggest the following guidelines in reporting studies of existing and novel mouse models of preterm birth.

Breeding and timing of gestational length

Because gestational length varies by mouse strain, it is extremely important to breed controls of the same genetic background when using transgenic models. We suggest two methods of breeding:

Timed breeding

One of the most accurate methods for estimating gestational length is to restrict the mating period [112]. Because hormonally receptive females usually mate within 1 h [114], pairing estrus-stage females with males for 2 to 4 h and then checking for the presence of a copulation plug allows for accurate assessment of gestational length. Alternatively, multiple breeding cages of unstaged females can be set up for a restricted time. The time at which the copulation plug is noted should be recorded as 0.0 dpc, and the following morning should be considered 0.5 dpc. We recommend caution in using timed-mated mice in preterm birth studies provided by a commercial supplier, as gestational length is likely to be affected by shipping, handling of the mice, potential quarantine, temperature changes, and alterations in light–dark cycles.

Overnight breeding

When mice cannot be bred for a restricted time period or less precision is acceptable, mice can be bred overnight. Place estrus-stage dams with stud males overnight, and check for the presence of a copulation plug before 8:00 am the next day. This method is

Figure 1. Skin permeability as a marker of maturity. In C57BL/6 mice, dye permeated mice at 17.0 dpc. By 18.0 dpc, dye was excluded on the dorsal side, but the pup remained blue on the ventral side. By 19 dpc, the skin was impermeable and faint blue stain was only noted on the ventral side. (Please see the online version for the color figure.)

effective because fertilization occurs around midnight, or the midpoint of the active period, in a 12-h dark/light cycle [115]. Thus, the time at which the plug is noted should be recorded as 0.5 dpc. Transgenic facilities commonly use this method and find that the majority of fertilized eggs harvested the morning after overnight mating are at the 0.5 dpc stage. This method will allow investigators to assign the time of conception (and thus the length of gestation) within a 12- to 18-h window.

Monitoring pregnancy outcomes

Timing and delivery complications

The most precise and efficient way to monitor delivery is to use an infrared video camera system. This allows an investigator to observe delivery of the first pup (which should be defined as the end of gestation), total duration of parturition, and subtle phenotypes such as dystocia, which can be ascertained by measuring the pup-to-pup interval (which averages 15 min for C57Bl/6; unpublished data). This method also allows detection of delivery even if the dam cannibalizes her litter.

Delivery outcomes

To establish a new model of preterm birth or validate an existing one, several informative characteristics should be recorded for each delivery: (1) litter size, because of the strong inverse correlation with gestational length [16, 116]; (2) offspring mortality rate, because any increase may indicate death related to prematurity; (3) pup crown-rump length [117]; and (4) pup weight. However, these characteristics are not definitive indicators of prematurity and may vary by strain. Lastly, we suggest use of reliable fetal growth markers that, preferably, are relevant to human fetal development. We describe two classes of such markers, involving skin and the lungs, in the next section.

Mouse fetal development markers

Here, we suggest two straightforward methods to assess fetal tissue maturation. The Institutional Animal Care and Use Committee at each respective institution approved all protocols performed for this review.

Skin barrier function

The skin forms a permeability barrier that regulates body temperature, prevents excess water loss, and prevents invasion by harmful pathogens. In humans, the epithelium fully develops its barrier function by late gestation (~34 weeks) [118]. Therefore, infants born before 30 weeks' gestation are at increased risk for infection and loss of temperature and fluid [119, 120]. The barrier develops when the outer layer of the epidermis forms the stratum corneum, which is composed of a tough insoluble cornified envelope that is "glued" together by a complex extracellular lipid matrix [121]. Barrier function develops similarly in mice late in gestation, beginning at 17 dpc and developing in a dorsal-to-ventral pattern until birth, when the barrier is fully formed. Thus, assessment of barrier function can indicate developmental stage of pups.

Methods to assess skin barrier maturation

Barrier function can be assessed by performing a whole-mount skin permeability assay as previously reported [122]. To perform this assay, dissect pups from the dams on the day of delivery, place them in a solution containing the blue dye 5-bromo-4-chloro-3- indolyl-β, D-galactopyranoside (X-gal), and gently nutate for 8 h to overnight at 37°C [122]. Wash samples twice with phosphate-buffered saline, fix overnight in 4% formaldehyde at 4°C, and store in phosphate-buffered saline at 4°C. Evaluate pups for blue dye penetration. Figure 1 shows examples of pups at different gestational stages subjected to this assay. For C57Bl6/J mice at 17 dpc, the skin barrier is immature and fetuses are uniformly blue. By 18 dpc, the skin on the dorsal side starts to cornify and becomes impermeable to the dye and is thus white, while the ventral side is still permeable and thus is blue. By late 18 to 19.0 dpc, the skin barrier has fully formed and the skin is impermeable to the dye, so the pup is almost completely white.

Skin maturation can also be assessed histologically [123]. To perform this assay, fix fetuses in 10% formalin, dehydrate them through an ethanol series, embed them in paraffin, cut 8 μm sections, mount the sections on glass slides, and perform routine hematoxylin and eosin (H&E) staining. Examine the skin for markers of maturation including thickness, stratification of the epidermal and dermal papillary layers, and hair follicle density. As shown in Figure 2, skin samples from 17 dpc pups have thin epidermal and dermal

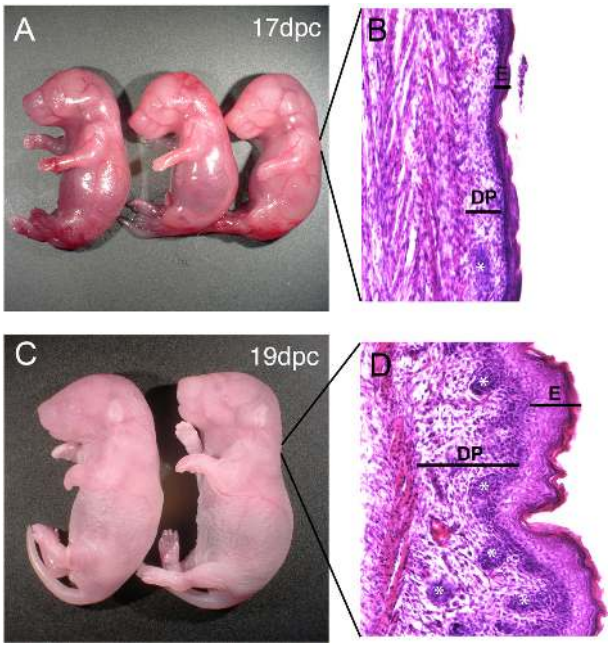

Figure 2. Skin histologic changes during gestation. In whole mount, the skin of 17 dpc CD-1 fetuses appeared more translucent (**A**) than that of 19 dpc fetuses (**C**). H&E-stained sections of dorsal skin from 17 dpc fetuses had thinner epidermal (**E**) and dermal papillary (DP) layers and contained fewer hair follicles (asterisks) than H&E-stained sections of skin from 19 dpc fetuses (**B** and **D**). (Please see the online version for the color figure.)

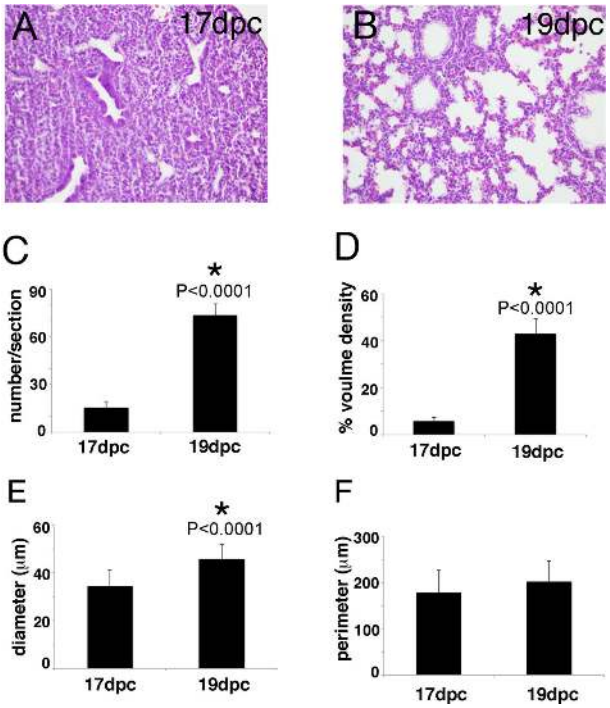

Figure 3. Lung morphometry as a marker of lung maturity. H&E stained lungs from 17 and 19 dpc CD-1 mouse fetuses (**A, B**). Lung morphometry measurements indicated that, compared to 17 dpc lungs, 19 dpc lungs contained more airspaces (**C**), had larger airspace volume density (airspace area divided by total lung area) (**D**), and had larger airspace diameter (**E**). However, airspace perimeter was similar in the two groups (**F**). n = 6 for both 17 and 19 dpc; *P-values, calculated by t-test; error bars represent SEM. (Please see the online version for the color figure.)

papillary regions and contain few hair follicles. In contrast, skin samples from 19 dpc pups have thick, stratified epidermal layers and thick papillary regions and contain multiple hair follicles.

Lung maturity markers

Lung development proceeds through markedly similar steps in mammals [124, 125]. This process begins with branching morphogenesis, in which the embryonic tracheal lung bud reiteratively branches to form large and small airways [126]. Branching morphogenesis is complete by mid-gestation in humans and mice and is followed by a period of mesenchymal thinning and epithelial differentiation. Finally, the lungs become alveolarized, during which alveolar septation expands the gas exchange structures of the lung [127]. In humans, this process begins at 36 weeks' gestation and continues into early childhood [128, 129]. In mice, alveolarization occurs entirely postnatally, beginning at postnatal day 4–5 and becoming fully mature by postnatal day 30. We describe two methods that can be used to assess the extent of lung mesenchymal thinning and epithelial differentiation as a marker of fetal maturity in mice [130].

Morphometric assessment of lung maturation

Morphometric assessment of lung airspaces reveals the degree of mesenchymal thinning that occurs after branching morphogenesis is complete and before alveolarization initiates. To evaluate lung morphology, dissect out fetal lungs, section them, H&E stain the sections, and view the lung sections at ×40 magnification (Figure 3A and B). Avoid fields containing large vessels or airways. Use computer-assisted morphometry (e.g. Image-Pro Plus software; Media Cybernetics) to count the number of airspaces and measure airspace diameter and airspace perimeter. Calculate airspace volume density by dividing the sum of the airspace area by the total area (Figure 3C–F). In our experience, the airspace perimeter is the least reliable measure of lung maturity because airspace shape becomes more variable as the mesenchyme thins [131].

Protein and mRNA markers of lung epithelial differentiation

Infants born prematurely, especially at <34 weeks' gestation, have pulmonary complications due to lung immaturity, and those infants that survive are at increased risk for long-term adverse pulmonary outcomes such as asthma, reduced lung function, and chronic lung disease [2, 5]. These pulmonary deficits are due, in part, to insufficient lung surfactant, composed of lipids and proteins that are secreted by fully differentiated type 2 alveolar epithelial cells. Surfactant reduces surface tension, helps maintain normal lung volumes, and is essential for normal breathing [132]. The four major protein components of surfactant are surfactant proteins B and C (SP-B and SP-C), which interact with phospholipids to improve surfactant dispersion at the air/liquid interface and prevent alveolar collapse [130, 133], and surfactant proteins A and D (SP-A and SP-D), which act as part of the innate immune system by opsonizing bacteria for clearance by pulmonary macrophages.

In mice, SP-A is expressed in the fetal lung late in gestation, becoming detectable in the amniotic fluid by 17.0 dpc and maximally expressed by 19.0 dpc [109]. Fetal SP-A is a particularly interesting marker of gestational lung maturity because it appears to help initiate parturition. Thus, detection of SP-A mRNA or protein in fetal lung or amniotic fluid could be used as a marker for fetal maturity in mice [109, 134]. SP-B, which is clinically administered to premature babies to treat surfactant deficiency, can likewise be used as a fetal

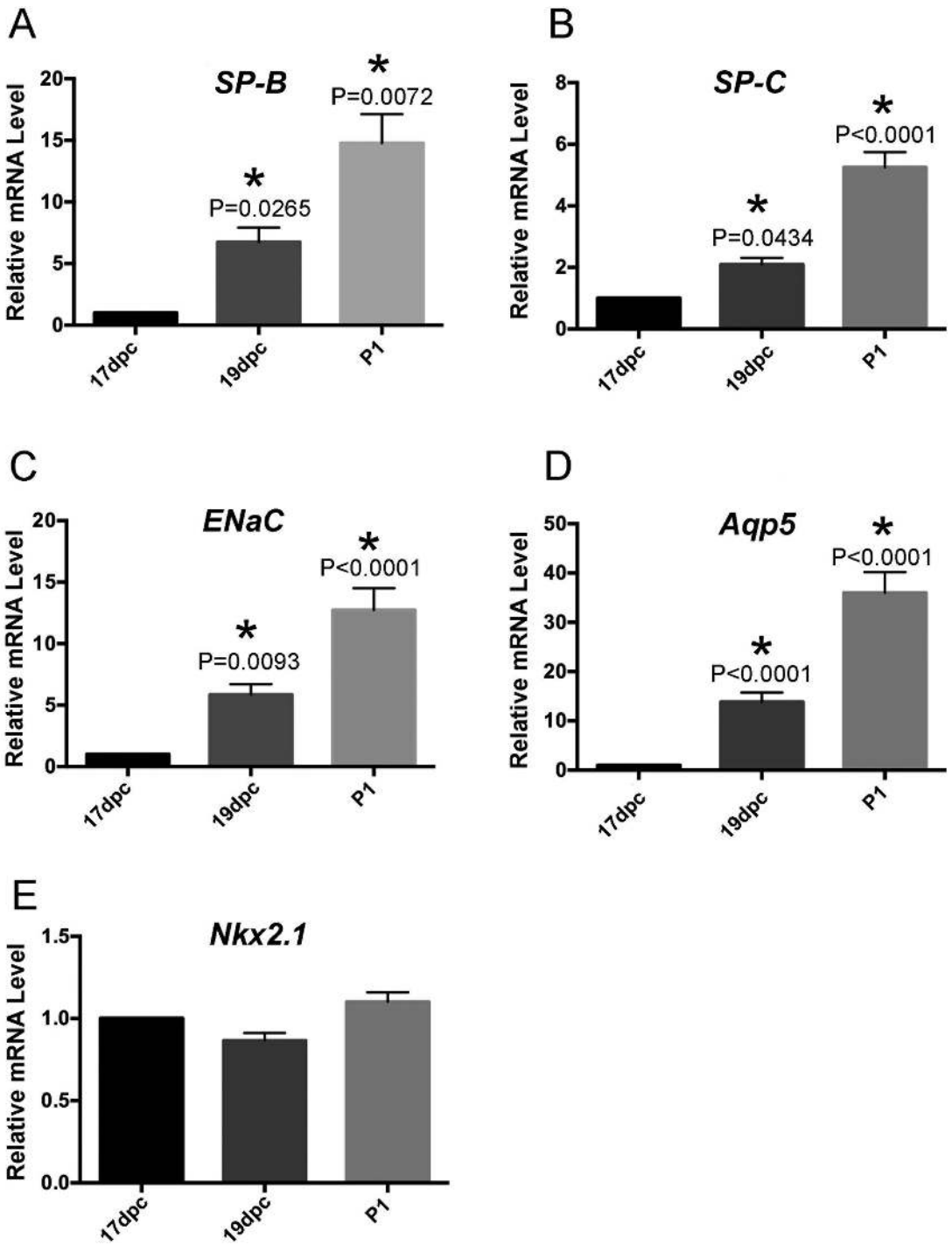

Figure 4. Gene expression as a marker of lung maturation. RNA isolated from pups of the indicated gestational ages was reverse transcribed to produce cDNA (SuperScript VILO kit; Invitrogen), and multiplex quantitative PCR reactions were performed with a StepOnePlus PCR System (Applied Biosystems) using the following FAM-labeled TaqMan Gene Expression assays (Applied Biosystems): SP-B Mm00455679_m1, ENac Mm00803386_m1, Aqp5 Mm00437578_m1, SP-C Mm00488144_m1, and Nkx2.1 Mm00447558_m1. The VIC-labeled housekeeping gene, 18S, was used as an internal control. Triplicate ΔΔCT values were generated for each sample. mRNA levels relative to dpc 17 mRNA were calculated by using the equation FC = $2^{-\Delta\Delta CT}$. n = 4 for each timepoint; *P-values, calculated by one-way ANOVA with Dunnett post hoc analysis, denote comparisons between indicated values and 17 dpc values; error bars represent SEM.

lung marker. As shown in Figure 4A, SP-B mRNA increases in the fetal lung by up to 5-fold between 17 and 19 dpc and by up to 10-fold at postnatal day 1. SP-C, a transmembrane protein expressed exclusively in alveolar type 2 cells postnatally, can similarly be used as a marker of lung maturity (Figure 4B)[135].

Two other proteins that can be used as markers of lung development are the water channel Aquaporin 5, which is expressed in alveolar type 1 cells and is thought to facilitate fluid absorption in the perinatal lung [136], and the epithelial sodium ion channel (ENaC). Two of the three ENaC subunits increase sharply in the mouse fetal

lung at late gestation [137]. As seen in Figure 4C and D, mRNA expression of ENaC and Aquaporin 5 increase in late gestation and can be used as markers of fetal lung maturity [135].

In addition to the above markers, panels of 50 or more genes have been evaluated as markers of fetal lung maturity in mice [130]. However, due to differences in mouse strain, detection methods, and reagents (primary antibodies, PCR primers), some of these proteins/genes may not be reproducible markers. For example, we found that NK2 Homeobox *1* (*Nkx2.1)*, an epithelial transcription factor critical for embryonic lung morphogenesis, was expressed at similar levels in 17 dpc, 19 dpc, and postnatal day 1 lungs. Therefore, we recommend that investigators evaluate multiple lung maturation markers. In addition, absolute levels of each marker are not sufficient to assess lung maturity and should always be compared to levels in full-term pups of the appropriate genotype and strain.

Conclusion

Researchers have used several mouse models to test hypotheses regarding preterm birth. These include models of genetic, infectious, noninfectious (sterile) inflammatory, environmental toxins, and endocrine etiologies. However, comparing data between studies is challenging given the lack of uniform criteria for defining preterm birth and assessing pup maturity. A recent review by Manuel et al. encourages investigators to develop better mouse models that are more consistent with the human etiologies of preterm birth [138]. In this review, we recommend that our field adopt standardized experimental and reporting guidelines to define preterm birth and use fetal developmental markers to distinguish between premature and mature pups. In addition to the skin and lung markers proposed here, we welcome other easy-to-use developmental markers. By standardizing our methods and reporting, mice should become an even more valuable model with which to study the important problems of preterm birth and the resulting neonatal co-morbidities.

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References

1. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller AB, Kinney M, Lawn J, Born Too Soon Preterm Birth Action Group. Born too soon: the global epidemiology of 15 million preterm births. *Reprod Health* 2013; **10**(Suppl 1):S2.
2. Lawn JE, Kinney M. Preterm birth: now the leading cause of child death worldwide. *Sci Transl Med* 2014; **6** (263):263ed21–263ed21.
3. Lawn JE, Kinney MV, Belizan JM, Mason EM, McDougall L, Larson J, Lackritz E, Friberg IK, Howson CP, Born Too Soon Preterm Birth ActionGroup. Born too soon: accelerating actions for prevention and care of 15 million newborns born too soon. *Reprod Health* 2013; **10**(Suppl 1):S6.
4. Liu L, Oza S, Hogan D, Chu Y, Perin J, Zhu J, Lawn JE, Cousens S, Mathers C, Black RE. Global, regional, and national causes of under-5 mortality in 2000–15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet North Am Ed* 2016; **388** (10063):3027–3035.
5. Raju TN, Pemberton VL, Saigal S, Blaisdell CJ, Moxey-Mims M, Buist S, Adults Born Preterm Conference Speakers and Discussants. Long-term healthcare outcomes of preterm birth: an executive summary of a conference sponsored by the National Institutes of Health. *J Pediatr* 2017; **181**:309–318.e1.
6. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet North Am Ed* 2008; **371** (9606):75–84.
7. Moutquin JM. Classification and heterogeneity of preterm birth. *BJOG* 2003; **110**(Suppl 20):30–33.
8. Esplin MS, Elovitz MA, Iams JD, Parker CB, Wapner RJ, Grobman WA, Simhan HN, Wing DA, Haas DM, Silver RM, Hoffman MK, Peaceman AM et al. Predictive accuracy of serial transvaginal cervical lengths and quantitative vaginal fetal fibronectin levels for spontaneous preterm birth among nulliparous women. *JAMA* 2017; **317** (10):1047–1056.
9. Mercer BM, Goldenberg RL, Das A, Moawad AH, Iams JD, Meis PJ, Copper RL, Johnson F, Thom E, McNellis D, Miodovnik M, Menard MK et al. The preterm prediction study: a clinical risk assessment system. *Am J Obstet Gynecol* 1996; **174** (6):1885–1895; discussion 1893–1885.
10. Son M, Miller ES. Predicting preterm birth: cervical length and fetal fibronectin. *Semin Perinatol* 2017; **41** (8):445–451.
11. Ratajczak CK, Muglia LJ. Insights into parturition biology from genetically altered mice. *Pediatr Res* 2008; **64** (6):581–589.
12. Elovitz MA, Mrinalini C. Animal models of preterm birth. *Trends Endocrinol Metab* 2004; **15** (10):479–487.
13. Brodt-Eppley J, Myatt L. Prostaglandin receptors in lower segment myometrium during gestation and labor. *Obstet Gynecol* 1999; **93**:89–93.
14. Cook JL, Zaragoza DB, Sung DH, Olson DM. Expression of myometrial activation and stimulation genes in a mouse model of preterm labor: myometrial activation, stimulation, and preterm labor. *Endocrinology* 2000; **141** (5):1718–1728.
15. Fuchs AR, Fuchs F, Husslein P, Soloff MS, Fernstrom MJ. Oxytocin receptors and human parturition: a dual role for oxytocin in the initiation of labor. *Science* 1982; **215** (4538):1396–1398.
16. Murray SA, Morgan JL, Kane C, Sharma Y, Heffner CS, Lake J, Donahue LR. Mouse gestation length is genetically determined. *PLoS One* 2010; **5** (8):e12418.
17. Bezold KY, Karjalainen MK, Hallman M, Teramo K, Muglia LJ. The genomics of preterm birth: from animal models to human studies. *Genome Med* 2013; **5** (4):34.
18. Martin JA, Hamilton BE, Osterman MJ, Driscoll AK, Mathews TJ. Births: Final Data for 2015. *Natl Vital Stat Rep* 2017; **66**:1.
19. Csapo AI, Pulkkinen MO, Ruttner B, Sauvage JP, Wiest WG. The significance of the human corpus luteum in pregnancy maintenance. *Am J Obstet Gynecol* 1972; **112** (8):1061–1067.
20. Cunningham FG, Williams JW. *Williams Obstetrics*. New York: McGraw-Hill Medical; 2010.
21. Andersson S, Minjarez D, Yost NP, Word RA. Estrogen and progesterone metabolism in the cervix during pregnancy and parturition. *J Clin Endocrinol Metab* 2008; **93** (6):2366–2374.
22. Nadeem L, Shynlova O, Mesiano S, Lye S. Progesterone via its type-A receptor promotes myometrial gap junction coupling. *Sci Rep* 2017; **7** (1):13357.
23. Mahendroo MS, Porter A, Russell DW, Word RA. The parturition defect in steroid 5alpha-reductase type 1 knockout mice is due to impaired cervical ripening. *Mol Endocrinol* 1999; **13**:981–992.
24. Norman JE, Bollapragada S, Yuan M, Nelson SM. Inflammatory pathways in the mechanism of parturition. *BMC Pregnancy Childbirth* 2007; **7**(Suppl 1):S7.
25. Galinsky R, Polglase GR, Hooper SB, Black MJ, Moss TJ. The consequences of chorioamnionitis: preterm birth and effects on development. *J Pregnancy* 2013; **2013**:1–11.
26. Ustun C, Kocak I, Baris S, Uzel A, Saltik F. Subclinical chorioamnionitis as an etiologic factor in preterm deliveries. *Int J Gynecol Obstet* 2001; **72**:109–115.
27. Higgins RD, Saade G, Polin RA, Grobman WA, Buhimschi IA, Watterberg K, Silver RM, Raju TN, Chorioamnionitis Workshop Participants. Evaluation and management of women and newborns with a maternal diagnosis of chorioamnionitis. *Obstet Gynecol* 2016; **127**:426–436.
28. Agrawal V, Hirsch E. Intrauterine infection and preterm labor. *Semin Fetal Neonatal Med* 2012; **17**:12–19.
29. Bastek JA, Gomez LM, Elovitz MA. The role of inflammation and infection in preterm birth. *Clin Perinatol* 2011; **38**:385–406.

30. Akgul Y, Word RA, Ensign LM, Yamaguchi Y, Lydon J, Hanes J, Mahendroo M. Hyaluronan in cervical epithelia protects against infection-mediated preterm birth. *J Clin Invest* 2014; **124**:5481–5489.
31. Hirsch E, Saotome I, Hirsh D. A model of intrauterine infection and preterm delivery in mice. *Am J Obstet Gynecol* 1995; **172**:1598–1603.
32. Kaga N, Katsuki Y, Obata M, Shibutani Y. Repeated administration of low-dose lipopolysaccharide induces preterm delivery in mice: a model for human preterm parturition and for assessment of the therapeutic ability of drugs against preterm delivery. *Am J Obstet Gynecol* 1996; **174**: 754–759.
33. Migale R, Herbert BR, Lee YS, Sykes L, Waddington SN, Peebles D, Hagberg H, Johnson MR, Bennett PR, MacIntyre DA. Specific lipopolysaccharide serotypes induce differential maternal and neonatal inflammatory responses in a murine model of preterm labor. *Am J Pathol* 2015; **185**:2390–2401.
34. Thaxton JE, Nevers TA, Sharma S. TLR-mediated preterm birth in response to pathogenic agents. *Infect Dis Obstet Gynecol* 2010; **2010**:8, Article ID 378472.
35. Elovitz MA, Wang Z, Chien EK, Rychlik DF, Phillippe M. A new model for inflammation-induced preterm birth. *Am J Pathol* 2003; **163**:2103–2111.
36. Gonzalez JM, Franzke CW, Yang F, Romero R, Girardi G. Complement activation triggers metalloproteinases release inducing cervical remodeling and preterm birth in mice. *Am J Pathol* 2011; **179**:838–849.
37. Ilievski V, Lu SJ, Hirsch E. Activation of toll-like receptors 2 or 3 and preterm delivery in the mouse. *Reprod Sci* 2007; **14**:315–320.
38. Kajikawa S, Kaga N, Futamura Y, Kakinuma C, Shibutani Y. Lipoteichoic acid induces preterm delivery in mice. *J Pharmacol Toxicol Methods* 1998; **39**:147–154.
39. Cardenas I, Mulla MJ, Myrtolli K, Sfakianaki AK, Norwitz ER, Tadesse S, Guller S, Abrahams VM. Nod1 activation by bacterial iE-DAP induces maternal-fetal inflammation and preterm labor. *J Immunol* 2011; **187**:980–986.
40. Berardi A, Cattelani C, Creti R, Berner R, Pietrangiolillo Z, Margarit I, Maione D, Ferrari F. Group B streptococcal infections in the newborn infant and the potential value of maternal vaccination. *Expert Rev Anti Infect Ther* 2015; **13**:1387–1399.
41. Bernardini R, Aufieri R, Detcheva A, Recchia S, Cicconi R, Amicosante M, Montesano C, Rossi P, Tchidjou HK, Petrunov B, Orefici G, Mattei M. Neonatal protection and preterm birth reduction following maternal group B streptococcus vaccination in a mouse model. *J Matern Fetal Neonatal Med* 2017; **30**:2844–2850.
42. Equils O, Moffatt-Blue C, Ishikawa TO, Simmons CF, Ilievski V, Hirsch E. Pretreatment with pancaspase inhibitor (Z-VAD-FMK) delays but does not prevent intraperitoneal heat-killed group B Streptococcus-induced preterm delivery in a pregnant mouse model. *Infect Dis Obstet Gynecol* 2009; **2009**:1–8.
43. Viscardi RM. Ureaplasma species: role in diseases of prematurity. *Clin Perinatol* 2010; **37**:393–409.
44. Sweeney EL, Dando SJ, Kallapur SG, Knox CL. The human ureaplasma species as causative agents of chorioamnionitis. *Clin Microbiol Rev* 2017; **30**:349–379.
45. Uchida K, Nakahira K, Mimura K, Shimizu T, De Seta F Wakimoto T, Kawai Y, Nomiyama M, Kuwano K, Guaschino S, Yanagihara I. Effects of Ureaplasma parvum lipoprotein multiple-banded antigen on pregnancy outcome in mice. *J Reprod Immunol* 2013; **100**:118–127.
46. Rours GI, Duijts L, Moll HA, Arends LR, de Groot R, Jaddoe VW, Hofman A, Steegers EA, Mackenbach JP, Ott A, Willemse HF, van der Zwaan EA et al. Chlamydia trachomatis infection during pregnancy associated with preterm delivery: a population-based prospective cohort study. *Eur J Epidemiol* 2011; **26**:493–502.
47. Pal S, Peterson EM, De La Maza LM. A murine model for the study of Chlamydia trachomatis genital infections during pregnancy. *Infect Immun* 1999; **67**:2607–2610.
48. Harris RE, Gilstrap LC, 3rd. Cystitis during pregnancy: a distinct clinical entity. *Obstet Gynecol* 1981; **57**:578–580.
49. Romero R, Oyarzun E, Mazor M, Sirtori M, Hobbins JC, Bracken M. Meta-analysis of the relationship between asymptomatic bacteriuria and preterm delivery/low birth weight. *Obstet Gynecol* 1989; **73**:576–582.
50. Kaul AK, Khan S, Martens MG, Crosson JT, Lupo VR, Kaul R. Experimental gestational pyelonephritis induces preterm births and low birth weights in C3H/HeJ mice. *Infect Immun* 1999; **67**:5958–5966.
51. Offenbacher S, Katz V, Fertik G, Collins J, Boyd D, Maynor G, McKaig R, Beck J. Periodontal infection as a possible risk factor for preterm low birth weight. *J Periodontol* 1996; **67**:1103–1113.
52. Horliana AC, Chambrone L, Foz AM, Artese HP, Rabelo Mde S, Pannuti CM, Romito GA. Dissemination of periodontal pathogens in the bloodstream after periodontal procedures: a systematic review. *PLoS One* 2014; **9**:e98271.
53. Leon R, Silva N, Ovalle A, Chaparro A, Ahumada A, Gajardo M, Martinez M, Gamonal J. Detection of porphyromonas gingivalis in the amniotic fluid in pregnant women with a diagnosis of threatened premature labor. *J Periodontol* 2007; **78**:1249–1255.
54. Katz J, Chegini N, Shiverick KT, Lamont RJ. Localization of P. gingivalis in preterm delivery placenta. *J Dent Res* 2009; **88**:575–578.
55. Ao M, Miyauchi M, Furusho H, Inubushi T, Kitagawa M, Nagasaki A, Sakamoto S, Kozai K, Takata T. Dental infection of *Porphyromonas gingivalis* induces preterm birth in mice. *PLoS One* 2015; **10**:e0137249.
56. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science* 2014; **345**:760–765.
57. Romero R, Miranda J, Chaiworapongsa T, Korzeniewski SJ, Chaemsaithong P, Gotsch F, Dong Z, Ahmed AI, Yoon BH, Hassan SS, Kim CJ, Yeo L. Prevalence and clinical significance of sterile intra-amniotic inflammation in patients with preterm labor and intact membranes. *Am J Reprod Immunol* 2014; **72**:458–474.
58. Heng YJ, Liong S, Permezel M, Rice GE, Di Quinzio MK, Georgiou HM. The interplay of the interleukin 1 system in pregnancy and labor. *Reprod Sci* 2014; **21**:122–130.
59. Bry K, Hallman M. Transforming growth factor-beta 2 prevents preterm delivery induced by interleukin-1 alpha and tumor necrosis factor-alpha in the rabbit. *Am J Obstet Gynecol* 1993; **168**:1318–1322.
60. Sadowsky DW, Adams KM, Gravett MG, Witkin SS, Novy MJ. Preterm labor is induced by intraamniotic infusions of interleukin-1beta and tumor necrosis factor-alpha but not by interleukin-6 or interleukin-8 in a nonhuman primate model. *Am J Obstet Gynecol* 2006; **195**: 1578–1589.
61. Romero R, Mazor M, Tartakovsky B. Systemic administration of interleukin-1 induces preterm parturition in mice. *Am J Obstet Gynecol* 1991; **165**:969–971.
62. Chen GY, Nunez G. Sterile inflammation: sensing and reacting to damage. *Nat Rev Immunol* 2010; **10**:826–837.
63. Romero R, Chaiworapongsa T, Alpay Savasan Z, Xu Y, Hussein Y, Dong Z, Kusanovic JP, Kim CJ, Hassan SS. Damage-associated molecular patterns (DAMPs) in preterm labor with intact membranes and preterm PROM: a study of the alarmin HMGB1. *J Matern Fetal Neonatal Med* 2011; **24**:1444–1455.
64. Gomez-Lopez N, Romero R, Plazyo O, Panaitescu B, Furcron AE, Miller D, Roumayah T, Flom E, Hassan SS. Intra-amniotic administration of HMGB1 induces spontaneous preterm labor and birth. *Am J Reprod Immunol* 2016; **75**:3–7.
65. Loctin J, Delost P. Mortality in premature mice at birth and during neonatal development. *Reprod Nutr Dévelop* 1983; **23**:293–301.
66. Barabas AP. Ehlers-Danlos syndrome: associated with prematurity and premature rupture of foetal membranes; possible increase in incidence. *BMJ* 1966; **2**:682–684.
67. Calmus ML, Macksoud EE, Tucker R, Iozzo RV, Lechner BE. A mouse model of spontaneous preterm birth based on the genetic ablation of biglycan and decorin. *Reproduction* 2011; **142**:183–194.
68. Peaceman AM, Andrews WW, Thorp JM, Cliver SP, Lukes A, Iams JD, Coultrip L, Eriksen N, Holbrook RH, Elliott J, Ingardia C, Pietrantoni M. Fetal fibronectin as a predictor of preterm birth in patients with symptoms: a multicenter trial. *Am J Obstet Gynecol* 1997; **177**:13–18.

69. Swamy GK, Simhan HN, Gammill HS, Heine RP. Clinical utility of fetal fibronectin for predicting preterm birth. *J Reprod Med* 2005; **50**:851–856.
70. Mogami H, Kishore AH, Shi H, Keller PW, Akgul Y, Word RA. Fetal fibronectin signaling induces matrix metalloproteases and cyclooxygenase-2 (COX-2) in amnion cells and preterm birth in mice. *J Biol Chem* 2013; **288**:1953–1966.
71. Mogami H, Keller PW, Shi H, Word RA. Effect of thrombin on human amnion mesenchymal cells, mouse fetal membranes, and preterm birth. *J Biol Chem* 2014; **289**:13295–13307.
72. Chigusa Y, Kishore AH, Mogami H, Word RA. Nrf2 activation inhibits effects of thrombin in human amnion cells and thrombin-induced preterm birth in mice. *J Clin Endocrinol Metab* 2016; **101**:2612–2621.
73. Tan H, Yi L, Rote NS, Hurd WW, Mesiano S. Progesterone receptor-A and -B have opposite effects on proinflammatory gene expression in human myometrial cells: implications for progesterone actions in human pregnancy and parturition. *J Clin Endocrinol Metab* 2012; **97**:E719–E730.
74. Condon JC, Jeyasuria P, Faust JM, Wilson JW, Mendelson CR. A decline in the levels of progesterone receptor coactivators in the pregnant uterus at term may antagonize progesterone receptor function and contribute to the initiation of parturition. *Proc Natl Acad Sci USA* 2003; **100**:9518–9523.
75. Dudley DJ, Branch DW, Edwin SS, Mitchell MD. Induction of preterm birth in mice by RU486. *Biol Reprod* 1996; **55**:992–995.
76. Holt R, Timmons BC, Akgul Y, Akins ML, Mahendroo M. The molecular mechanisms of cervical ripening differ between term and preterm birth. *Endocrinology* 2011; **152**:1036–1046.
77. Nallasamy S, Akins M, Tetreault B, Luby-Phelps K, Mahendroo M. Distinct reorganization of collagen architecture in lipopolysaccharide - mediated premature cervical remodeling. *Biol Reprod* 2018; **98**:63–74.
78. Norwitz E, Robinson J, Repke J. Obstetrics; normal and problem pregnancies. In: Gabbe SG, Niebyl JR, Simpson JL (eds.), *Obstetrics: Normal and Problem Pregnancies*. 4th ed. New York: Churchill Livingstone; 2002: 353–394.
79. Timmons BC, Reese J, Socrate S, Ehinger N, Paria BC, Milne GL, Akins ML, Auchus RJ, McIntire D, House M, Mahendroo M. Prostaglandins are essential for cervical ripening in LPS-mediated preterm birth but not term or antiprogestin-driven preterm ripening. *Endocrinology* 2014; **155**:287–298.
80. Roizen JD, Asada M, Tong M, Tai HH, Muglia LJ. Preterm birth without progesterone withdrawal in 15-hydroxyprostaglandin dehydrogenase hypomorphic mice. *Mol Endocrinol* 2008; **22**:105–112.
81. Kishore AH, Liang H, Kanchwala M, Xing C, Ganesh T, Akgul Y, Posner B, Ready JM, Markowitz SD, Word RA. Prostaglandin dehydrogenase is a target for successful induction of cervical ripening. *Proc Natl Acad Sci USA* 2017; **114**:E6427–E6436.
82. Kurtzman JT, Spinnato JA, Goldsmith LJ, Zimmerman MJ, Klem M, Lei ZM, Rao CV. Human chorionic gonadotropin exhibits potent inhibition of preterm delivery in a small animal model. *Am J Obstet Gynecol* 1999; **181**:853–857.
83. Menon R, Bonney EA, Condon J, Mesiano S, Taylor RN. Novel concepts on pregnancy clocks and alarms: redundancy and synergy in human parturition. *Hum Reprod Update* 2016; **22**:535–560.
84. Deng W, Cha J, Yuan J, Haraguchi H, Bartos A, Leishman E, Viollet B, Bradshaw HB, Hirota Y, Dey SK. p53 coordinates decidual sestrin 2/AMPK/mTORC1 signaling to govern parturition timing. *J Clin Invest* 2016; **126**:2941–2954.
85. Hirota Y, Cha J, Yoshie M, Daikoku T, Dey SK. Heightened uterine mammalian target of rapamycin complex 1 (mTORC1) signaling provokes preterm birth in mice. *Proc Natl Acad Sci USA* 2011; **108**:18073–18078.
86. Hirota Y, Daikoku T, Tranguch S, Xie H, Bradshaw HB, Dey SK. Uterine-specific p53 deficiency confers premature uterine senescence and promotes preterm birth in mice. *J Clin Invest* 2010; **120**:803–815.
87. Cha J, Bartos A, Egashira M, Haraguchi H, Saito-Fujita T, Leishman E, Bradshaw H, Dey SK, Hirota Y. Combinatory approaches prevent preterm birth profoundly exacerbated by gene-environment interactions. *J Clin Invest* 2013; **123**:4063–4075.
88. Jeyasuria P, Wetzel J, Bradley M, Subedi K, Condon JC. Progesterone-regulated caspase 3 action in the mouse may play a role in uterine quiescence during pregnancy through fragmentation of uterine myocyte contractile proteins. *Biol Reprod* 2009; **80**:928–934.
89. Kyathanahalli C, Organ K, Moreci RS, Anamthathmakula P, Hassan SS, Caritis SN, Jeyasuria P, Condon JC. Uterine endoplasmic reticulum stress-unfolded protein response regulation of gestational length is caspase-3 and -7–dependent. *Proc Natl Acad Sci USA* 2015; **112**:14090–14095.
90. Dennedy MC, Friel AM, Houlihan DD, Broderick VM, Smith T, Morrison JJ. Cannabinoids and the human uterus during pregnancy. *Am J Obstet Gynecol* 2004; **190**:2–9; discussion 3A.
91. Habayeb OM, Taylor AH, Evans MD, Cooke MS, Taylor DJ, Bell SC, Konje JC. Plasma levels of the endocannabinoid anandamide in women—a potential role in pregnancy maintenance and labor? *J Clin Endocrinol Metab* 2004; **89**:5482–5487.
92. Cota D, Steiner MA, Marsicano G, Cervino C, Herman JP, Grubler Y, Stalla J, Pasquali R, Lutz B, Stalla GK, Pagotto U. Requirement of cannabinoid receptor type 1 for the basal modulation of hypothalamic-pituitary-adrenal axis function. *Endocrinology* 2007; **148**:1574–1581.
93. McLean M, Bisits A, Davies J, Woods R, Lowry P, Smith R. A placental clock controlling the length of human pregnancy. *Nat Med* 1995; **1**:460–463.
94. Karalis K, Goodwin G, Majzoub JA. Cortisol blockade of progesterone: a possible molecular mechanism involved in the initiation of human labor. *Nat Med* 1996; **2**:556–560.
95. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 1990; **346**:561–564.
96. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993; **365**:61–65.
97. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 1992; **258**:1946–1949.
98. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K. 2-Arachidonoylgylcerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* 1995; **215**:89–97.
99. Wang H, Xie H, Dey SK. Loss of cannabinoid receptor CB1 induces preterm birth. *PLoS One* 2008; **3**:e3320.
100. Kramer MS, Kahn SR, Rozen R, Evans R, Platt RW, Chen MF, Goulet L, Seguin L, Dassa C, Lydon J, McNamara H, Dahhou M et al. Vasculopathic and thrombophilic risk factors for spontaneous preterm birth. *Int J Epidemiol* 2009; **38**:715–723.
101. Maron BA, Loscalzo J. The treatment of hyperhomocysteinemia. *Annu Rev Med* 2009; **60**:39–54.
102. Sonne SR, Bhalla VK, Barman SA, White RE, Zhu S, Newman TM, Prasad PD, Smith SB, Offermanns S, Ganapathy V. Hyperhomocysteinemia is detrimental to pregnancy in mice and is associated with preterm birth. *Biochim Biophys Acta*2013; **1832**:1149–1158.
103. Wallace JL, Aland KL, Blatt K, Moore E, DeFranco EA. Modifying the risk of recurrent preterm birth: influence of trimester-specific changes in smoking behaviors. *Am J Obstet Gynecol* 2017; **216**: 310 e311–310 e318.
104. Ng SP, Zelikoff JT. The effects of prenatal exposure of mice to cigarette smoke on offspring immune parameters. *J Toxicol Environ Health A* 2008; **71**:445–453.
105. Bruner-Tran KL, Osteen KG. Developmental exposure to TCDD reduces fertility and negatively affects pregnancy outcomes across multiple generations. *Reprod Toxicol* 2011; **31**:344–350.
106. Ding T, Lambert LA, Aronoff DM, Osteen KG, Bruner-Tran KL. Sex-dependent influence of developmental toxicant exposure on group b Streptococcus-mediated preterm birth in a murine model. *Reprod Sci* 2018; **25**:662–673.

107. Tiboni GM, Giampietro F. Inhibition of nitric oxide synthesis causes preterm delivery in the mouse. *Hum Reprod* 2000; **15**:1838–1842.
108. Tiboni GM, Giampietro F, Lamonaca D. The soluble guanylate cyclase inhibitor methylene blue evokes preterm delivery and fetal growth restriction in a mouse model. *In Vivo* 2001; **15**:333–337.
109. Condon JC, Jeyasuria P, Faust JM, Mendelson CR. Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. *Proc Natl Acad Sci USA* 2004; **101**:4978–4983.
110. Zhang WS, Xie QS, Wu XH, Liang QH. Neuromedin B and its receptor induce labor onset and are associated with the RELA (NFKB P65)/IL6 pathway in pregnant mice. *Biol Reprod* 2011; **84**:113–117.
111. Cook JL, Randall CL. Early onset of parturition induced by acute alcohol exposure in C57BL/6J mice: role of uterine PGE and PGF2a. *Reprod Fertil Dev* 1997; **9**:815–823.
112. Endo A, Watanabe T. Interlitter variability in fetal body weight in mouse offspring from continuous, overnight, and short-period matings. *Teratology* 1988; **37**:63–67.
113. Beniest-Noirot E. Analyse du comportement dit maternal chez la souris. *Cent Nat Rech Sci Monogr Franc Psychol*. 1958; No. 1.
114. Wimer RE, Fuller JL. Patterns of Behavior In: Green EL (ed.) *Biology of the Laboratory Mouse*. New York: Dover Publications; 1966: 629–653.
115. Behringer R. *Manipulating the Mouse Embryo: a Laboratory Manual*. New York: Cold Spring Harbor Laboratory Press; 2014.
116. Biggers JD, Curnow RN, Finn CA, McLaren A. Regulation of the gestation period in mice. *Reproduction* 1963; **6**:125–138.
117. McCoy AM, Herington JL, Stouch AN, Mukherjee AB, Lakhdari O, Blackwell TS, Prince LS. IKKβ activation in the fetal lung mesenchyme alters lung vascular development but not airway morphogenesis. *Am J Pathol* 2017; **187**:2635–2644.
118. Evans NJ, Rutter N. Development of the epidermis in the newborn. *Neonatology* 1986; **49**:74–80.
119. Vernon HJ, Lane AT, Wischerath LJ, Davis JM, Menegus MA. Semipermeable dressing and transepidermal water loss in premature infants. *Pediatrics* 1990; **86**:357–362.
120. Cartlidge P. The epidermal barrier. *Semin Neonatol* 2000; **5**:273–280.
121. Menon GK, Cleary GW, Lane ME. The structure and function of the stratum corneum. *Int J Pharm* 2012; **435**:3–9.
122. Hardman MJ, Sisi P, Banbury DN, Byrne C. Patterned acquisition of skin barrier function during development. *Development* 1998; **125**:1541–1552.
123. Forni MF, Trombetta-Lima M, Sogayar MC. Stem cells in embryonic skin development. *Biol Res* 2012; **45**:215–222.
124. Pringle KC. Human fetal lung development and related animal models. *Clin Obstet Gynecol* 1986; **29**:502–513.
125. Thurlbeck WM. Postnatal growth and development of the lung. *Am Rev Respir Dis* 1975; **111**:803–844.
126. Metzger RJ, Klein OD, Martin GR, Krasnow MA. The branching programme of mouse lung development. *Nature* 2008; **453**:745–750.
127. Bourbon J, Boucherat O, Chailley-Heu B, Delacourt C. Control mechanisms of lung alveolar development and their disorders in bronchopulmonary dysplasia. *Pediatr Res* 2005; **57**:38R–46R.
128. Burri PH. Fetal and postnatal development of the lung. *Annu Rev Physiol* 1984; **46**:617–628.
129. Morrisey EE, Hogan BL. Preparing for the first breath: genetic and cellular mechanisms in lung development. *Dev Cell* 2010; **18**:8–23.
130. Besnard V, Wert SE, Ikegami M, Xu Y, Heffner C, Murray SA, Donahue LR, Whitsett JA. Maternal synchronization of gestational length and lung maturation. *PLoS One* 2011; **6**:e26682.
131. Plosa EJ, Young LR, Gulleman PM, Polosukhin VV, Zaynagetdinov R, Benjamin JT, Im AM, van der Meer R, Gleaves LA, Bulus N, Han W, Prince LS et al. Epithelial 1 integrin is required for lung branching morphogenesis and alveolarization. *Development* 2014; **141**:4751–4762.
132. Clements JA. Pulmonary surfactant. *Am Rev Respir Dis* 1970; **101**:984–990.
133. Mendelson CR, Chen C, Boggaram V, Zacharias C, Snyder JM. Regulation of the synthesis of the major surfactant apoprotein in fetal rabbit lung tissue. *J Biol Chem* 1986; **261**:9938–9943.
134. Mendelson CR, Condon JC. New insights into the molecular endocrinology of parturition. *J Steroid Biochem Mol Biol* 2005; **93**:113–119.
135. Shelton EL, Waleh N, Plosa EJ, Benjamin JT, Milne GL, Hooper CW, Ehinger NJ, Poole S, Brown N, Seidner S, McCurnin D, Reese JJ et al. Effects of antenatal betamethasone on preterm human and mouse ductus arteriosus: comparison with baboon data. *Pediatr Res* 2018; in press.
136. Ma T, Fukuda N, Song Y, Matthay MA, Verkman AS. Lung fluid transport in aquaporin-5 knockout mice. *J Clin Invest* 2000; **105**:93–100.
137. Talbot CL, Bosworth DG, Briley EL, Fenstermacher DA, Boucher RC, Gabriel SE, Barker PM. Quantitation and localization of ENaC subunit expression in fetal, newborn, and adult mouse lung. *Am J Respir Cell Mol Biol* 1999; **20**:398–406.
138. Manuel CR, Ashby CR, Reznik SE. Discrepancies in animal models of preterm birth. *Curr Pharm Des* 2017; **23**:6142–6148.
139. Sun X, Deng W, Li Y, Tang S, Leishman E, Bradshaw HB, Dey SK. Sustained endocannabinoid signaling compromises decidual function and promotes inflammation-induced preterm birth. *J Biol Chem* 2016; **291**:8231–8240.
140. Guo Y, Ma Z, Kou H, Sun R, Yang H, Smith CV, Zheng J, Wang H. Synergistic effects of pyrrolizidine alkaloids and lipopolysaccharide on preterm delivery and intrauterine fetal death in mice. *Toxicol Lett* 2013; **221**:212–218.
141. Edey LF, O'Dea KP, Herbert BR, Hua R, Waddington SN, MacIntyre DA, Bennett PR, Takata M, Johnson MR. The local and systemic immune response to intrauterine LPS in the prepartum mouse. *Biol Reprod* 2016; **95**:125–125.
142. Chin PY, Dorian CL, Hutchinson MR, Olson DM, Rice KC, Moldenhauer LM, Robertson SA. Novel Toll-like receptor-4 antagonist (+)-naloxone protects mice from inflammation-induced preterm birth. *Sci Rep* 2016; **6**:36112.
143. Gomez-Lopez N, Romero R, Arenas-Hernandez M, Panaitescu B, Garcia-Flores V, Mial TN, Sahi A, Hassan SS. Intra-amniotic administration of lipopolysaccharide induces spontaneous preterm labor and birth in the absence of a body temperature change. *J Matern Fetal Neonatal Med* 2018; **31**:439–446.
144. Stelloh C, Allen KP, Mattson DL, Lerch-Gaggl A, Reddy S, El-Meanawy A. Prematurity in mice leads to reduction in nephron number, hypertension, and proteinuria. *Transl Res* 2012; **159**:80–89.
145. Ng SP, Steinetz BG, Lasano SG, Zelikoff JT. Hormonal changes accompanying cigarette smoke-induced preterm births in a mouse model. *Exp Biol Med (Maywood)* 2006; **231**:1403–1409.