

Washington University School of Medicine Digital Commons@Becker

Open Access Publications

2010

Preventing preterm birth: the past limitations and new potential of animal models

Christine K. Ratajczak

Washington University School of Medicine in St. Louis

Justin C. Fay

Washington University School of Medicine in St. Louis

Louis J. Muglia

Vanderbilt University School of Medicine

Follow this and additional works at: http://digitalcommons.wustl.edu/open_access_pubs

Recommended Citation

Ratajczak, Christine K.; Fay, Justin C.; and Muglia, Louis J., "Preventing preterm birth: the past limitations and new potential of animal models." *Disease Models & Mechanisms*.3,7-8. 407-414. (2010).

http://digitalcommons.wustl.edu/open_access_pubs/1435

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact engeszer@wustl.edu.

Preventing preterm birth: the past limitations and new potential of animal models

Christine K. Ratajczak¹, Justin C. Fay² and Louis J. Muglia^{3,*}

The high rate of preterm birth in the USA and many other countries is a potential target for improving children's immediate health and reducing the medical problems they face as adults. The acute complications for infants born prematurely often require intensive care management and are followed by long-lasting cognitive, sensory, motor, and cardiovascular deficits that substantially limit adult capabilities and survival. The inability to effectively reduce preterm birth stems from the failure to understand normal mechanisms of parturition in humans. Although studies from several model organisms help define the physiology of maintenance and termination of pregnancy, there are fundamental differences between species. For example, species regulate their production of progesterone, the crucial hormone in sustaining pregnancy, differently. This limits the extent to which models can provide meaningful information about the physiological mechanisms of human gestation. The growing wealth of sequenced mammalian genomes, computational comparative genomic tools and systems biology approaches provides new potential to utilize the divergence of DNA sequences and physiology between species to understand the genetic underpinnings of preterm birth.

Preterm birth: an increasing public health concern

Three million infants die before their first birthday as the result of being born prematurely (Ahman and Zupan, 2007). According to global statistics, the greatest cause of infant mortality in the USA is preterm birth, which is the birth of a child after less than 37 weeks of completed gestation, rather than the typical 40 weeks (Committee on Understanding Premature Birth and Assuring Healthy Outcomes Board on Health Sciences Policy, 2006). In addition to a high risk for neonatal death, preterm infants have an increased incidence of chronic complications including intellectual disability, respiratory illness, and vision and hearing impairment. These pathologies make prematurity a leading cause of disability adjusted life-years (DALYs), as they arise at the earliest stages of life (Murray and Lopez, 1997).

In 2007, 12.7% of all births in the USA were preterm, an increase of approximately 20% since 1990 (Heron et al., 2010). The rate of preterm birth in black women in the USA remains nearly twofold greater than for white women with otherwise-similar demographic characteristics (Martin et al., 2009). This difference represents one of the largest racial disparities in national health. Preterm birth is a complex multifactorial process associated with diverse pathogenetic mechanisms including infection, maternal stress and changes in obstetric practices, such as indications for induced delivery and artificial reproductive technologies. Birth, or parturition, which is the process of giving birth, involves a complex switch of the uterus from a static and docile environment to the generator of contractile force sufficient to expel a baby. Little is known about the factors that regulate this dynamic switch. Approximately one half of all

preterm births are idiopathic (Muglia and Katz, 2010) with no recognized precipitating factor. Increased insight into the etiology of spontaneous idiopathic preterm birth is necessary to effectively model and address this rising public health concern.

Risk factors for preterm birth

Genetic factors influence preterm birth. A family history of preterm birth is a key risk factor, such that women whose mothers and sisters have had a preterm delivery are more likely to enter labor prematurely (Porter et al., 1997; Winkvist et al., 1998). A small but significant association also links the paternal genome to preterm labor; this is probably because of the influence of paternally contributed fetal genes, as demonstrated by segregation analyses and twin studies (Kistka et al., 2008; Plunkett et al., 2009). Rates of preterm birth in specific ethnic groups, with genetic variation arising from geographic history, also indicate a role for genetics in preterm birth. For example, a higher incidence of preterm birth has been demonstrated when either the mother (Adams et al., 2000; Kistka et al., 2007) or father (Palomar et al., 2007) is African American. Several studies indicate that environmental risk factors are not sufficient to explain racial differences in preterm birth (e.g. Goldenberg et al., 1996; Kistka et al., 2007). These population-based findings indicate a role for genetic variation in human birth timing and the risk for prematurity. Clearly, identification of the genetic factors underlying preterm birth would help researchers understand and model the disease.

Initial attempts to reveal the specific genetic variants that contribute to preterm birth involved candidate gene association studies comparing women giving birth to premature infants, or the infants themselves, with women or infants experiencing term births. Several common risk-conferring polymorphisms in inflammation, connective tissue remodeling and detoxification enzymes were identified (Crider et al., 2005;

¹Molecular Cell Biology Program, Washington University, St Louis, MO 63110, USA

²Department of Genetics and Center for Genome Sciences, Washington University, St Louis, MO 63108, USA

³Department of Pediatrics, Vanderbilt University School of Medicine and Monroe Carell Jr Children's Hospital at Vanderbilt, Nashville, TN 37215, USA

*Author for correspondence (louis.muglia@vanderbilt.edu)

Plunkett and Muglia, 2008). The association of these polymorphisms with disease is small and variable, but one emerging finding links racial differences in preterm birth frequency with a functional polymorphism in the promoter of a chaperone that is essential for collagen synthesis, SERPINH1. The mutation associated with preterm birth reduces the activity of the SERPINH1 promoter (Wang et al., 2006). This variant is increased in populations of African descent, and is associated with a 3.22-fold increase (95% confidence interval: 1.50-7.22) in the incidence of preterm birth associated with premature rupture of the fetal membranes.

Spontaneous idiopathic preterm birth may reflect acceleration of the normal gestational clock. Microarray data from uterine tissue reveals similar gene regulation patterns among women who labor prematurely and those who do not labor until their infants are full term (Bethin et al., 2003). The lack of distinguishing pathways between preterm and full-term gestation suggests that normal pathways may be accelerated in preterm birth. Thus, factors involved in normal pathway timing may be targets for predicting or preventing preterm birth.

Infection deregulates the normal timing of parturition mechanisms. Mothers who deliver preterm infants, particularly at very early gestational ages, display an increased frequency of colonization with possibly pathogenic microorganisms, even when they do not demonstrate overt evidence of infection (Watts et al., 1992; Onderdonk et al., 2008). Several animal models have been utilized to explore the normal timing mechanisms of parturition and the consequences of infection to elucidate pathways for preterm birth. Although they do not recapitulate the human disease in full, they do offer valuable insights.

Animal studies of parturition

The use of humans in controlled studies on parturition is severely limited by ethical considerations. However, studies of other animals, notably sheep and mice, have made key contributions to the understanding of parturition. The use of each of these model systems has its own advantages and disadvantages.

Sheep studies

Sheep have been a relevant and valuable model for preterm birth. Their relatively

Case study

J.M. was the 1000-gram product of a 26-week gestation pregnancy to an 18-year-old mother without previous pregnancies. This pregnancy was uneventful until the onset of preterm labor that could not be controlled with medical therapy. J.M. was delivered vaginally in breech position, and required cardiopulmonary resuscitation in the delivery room. After placement of an endotracheal tube for assisted lung ventilation, she was transferred to the neonatal intensive care unit for further management. There, J.M. suffered several complications including: (1) mechanical ventilation for 7 weeks, after which chronic steroids and supplemental oxygen were needed; (2) cerebral bleeding (intraventricular hemorrhage) causing hydrocephalus, which was treated with spinal taps and ultimately needed placement of a ventricular shunt to drain fluid; (3) retinopathy of prematurity that significantly impaired vision; (4) cognitive and motor deficits in childhood; and (5) sustained impairment in growth. These deficits are common following preterm birth. Although neonatal care continues to improve outcomes, the potential for preventing preterm birth offers the greatest hope for the future health of children.

large size allows for specific manipulation of either fetus or pregnant ewe. Also, the gestation length of humans is closer to that of sheep than other common models like mice and rats. Both humans and sheep experience a luteal-placental shift during pregnancy (Challis et al., 2000), when the responsibility for steroid hormone production shifts from the corpus luteum to the placenta as gestation progresses. As with humans, sheep have a small number of offspring, with typically one or two fetuses per gestation. However, sheep have a bicornuate uterus as opposed to the pyriform, unicornuate uterus in humans.

Sheep studies demonstrate fetal regulation of parturition events. In the 1960s, Liggins and colleagues (Liggins et al., 1967; Liggins, 1968) demonstrated the importance of the fetal hypothalamic-pituitary-adrenal (HPA) axis in determining birth timing in the sheep. During late gestation, the levels of adrenocorticotrophic hormone (ACTH) and cortisol in sheep fetal plasma increase (Challis et al., 2000). Rising cortisol levels cause a change in how pregnenolone is metabolized in the placenta; at this stage pregnenolone that had previously become progesterone is converted to 17- α -hydroxypregnenolone and 17- β -estradiol. The resulting decrease in circulating maternal progesterone levels, and rise in circulating maternal estrogen, triggers increased uterine levels of the contractile agonist prostaglandin F_{2 α} (PGF_{2 α}) and subsequent uterine contraction, leading to parturition. Rising cortisol levels also promote maturation of fetal lungs and other organs just before birth. This is evident in the viability of fetuses born prematurely owing to fetal infusion with ACTH or cortisol (Liggins, 1968). Thus, fetal ACTH and cortisol from the fetus help coordinate parturition events with fetal maturity.

Kitts and colleagues also used embryo transfer in sheep to establish that birth timing is determined by the shorter gestation-length twin (Kitts et al., 1984). These results further demonstrate the important role of the fetus in precipitating changes that are required for the start of parturition in sheep.

Mouse studies

Despite several important differences in mouse and human pregnancy (Fig. 1), the genetic tractability of the mouse makes it an attractive mammalian model system (Ratajczak and Muglia, 2008). This allows for the generation of knockout models to examine the role of genes that might influence parturition. Mice also have a short gestation time of 19.5 days, which is convenient for experimentation. However, there are distinct differences between mouse and human reproductive physiology. Mice have a bicornuate uterus and a large litter size, averaging between six and ten pups (Fig. 1). Furthermore, whereas humans experience a luteal-placental shift, the mouse corpora lutea are responsible for steroid hormone production throughout gestation (for a review, see Ratajczak and Muglia, 2008).

As with sheep, mouse parturition involves progesterone withdrawal. Whereas in the sheep, the placenta has a role in steroid hormone production during pregnancy, the corpora lutea of the mouse ovaries are responsible for steroid production throughout pregnancy. In the mouse, PGF_{2 α} stimulates luteolysis, the structural and functional degradation of the corpora lutea. Levels of circulating maternal progesterone then drop and uterine contractility increases. Accordingly, ovariectomy induces preterm labor through surgical removal of the site of endogenous progesterone production, whereas administration

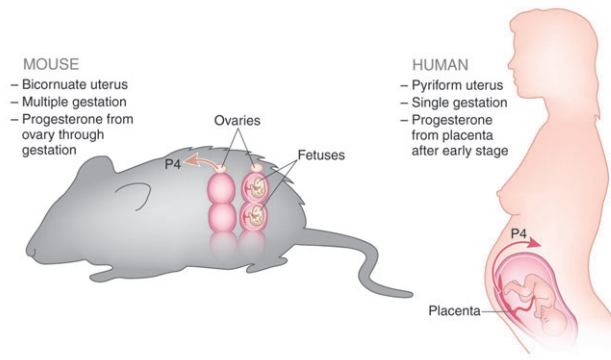


Fig. 1. Comparison of human and mouse pregnancy. Fundamental differences in the anatomy and physiology between these species include uterine shape, number of embryos, and the site of generation of progesterone in late gestation (P4).

and $\text{PGF}_{2\alpha}$ appear to diminish uterine contractility enough to prolong labor.

Studies of Cx43-deficient mice demonstrate its importance in parturition. Cx43 coordinates myometrial cells to produce contractions during labor. Mice with a global deficiency for Cx43 die during the neonatal period (Reaume et al., 1995). Therefore, mice that are deficient for Cx43 specifically in smooth muscle tissues, including the myometrium, were used to examine the role of Cx43 in parturition (Doring et al., 2006). These mice experienced somewhat-delayed parturition despite normal Oxt_r and FP upregulation and progesterone withdrawal (Doring et al., 2006).

Genetically altered mice also provide insight into the process of cervical ripening. Toward the end of gestation, softening and remodeling of the cervix is important in readying the birth canal for labor (Lepert, 1995). Mice deficient for the hormone relaxin or its receptor (LGR7) exhibit a low-penetrance, nonproductive labor phenotype (Zhao et al., 1999; Krajnc-Franken et al., 2004). This is probably caused by defects in cervical ripening, as relaxin-deficient females have more collagen in the pubic symphysis, cervix and vagina one day before term (Zhao et al., 2000). Mice that are deficient for 5 α -reductase type 1 (5 α -R1), a progesterone-metabolizing enzyme, also have increased cervical collagen at term (Mahendroo et al., 1999). 5 α -R1^{-/-} females experience prolonged labor 2-3 days post-term (Mahendroo et al., 1996). Their serum progesterone at term decreases and progesterone levels in the uterus and cervix are high (Mahendroo et al., 1999). Ovariectomy, progesterone receptor antagonists, oxytocin or relaxin can all reverse the labor phenotype in these females (Mahendroo et al., 1996; Mahendroo et al., 1999). Thus, genetic mutation may accelerate parturition rather than impair cervical ripening.

Approximately 25% of human preterm births are associated with infection (Muglia and Katz, 2010). Experiments in normal and genetically altered mice have been used to elucidate the role of cytokines and inflammation in this process. Intraperitoneal administration of lipopolysaccharide (LPS), a component of the cell membrane of Gram-negative bacteria that activates Toll-like receptor 4 (TLR4) and a host proinflammatory response, causes preterm labor in mice (Fidel et al., 1998; Gross et al., 2000).

of exogenous progesterone delays the commencement of labor in mice (Skarnes and Harper, 1972). The importance of progesterone withdrawal for murine labor has been highlighted by the observed phenotypes of several genetically modified mouse lines. Mice that are deficient in 20- α -hydroxysteroid dehydrogenase, an enzyme that converts progesterone to an inactive metabolite and that is expressed in the ovary of term mice (Wiest, 1968), exhibit no progesterone withdrawal and a failure to initiate labor at term (Piekorz et al., 2005). Similarly, mice that are deficient in the $\text{PGF}_{2\alpha}$ receptor (FP) do not experience a serum progesterone withdrawal at term and fail to initiate parturition (Sugimoto et al., 1997). Mice that are deficient in enzymes involved in the synthesis of prostaglandins, such as cytoplasmic phospholipase A₂ and cyclooxygenase-1, also experience delayed labor that can be reversed by progesterone receptor antagonist or exogenous $\text{PGF}_{2\alpha}$ (Langenbach et al., 1995; Bonventre et al., 1997; Uozumi et al., 1997; Gross et al., 1998).

In addition to their role in luteolysis, prostaglandins serve as a uterine contractile agonist at parturition. The importance of this additional role of prostaglandins is demonstrated in studies of mice with reduced 15-hydroxyprostaglandin dehydrogenase (15-HPGD). This enzyme metabolizes $\text{PGF}_{2\alpha}$ and prostaglandin E₂ (PGE_2) (Okita and Okita, 1996; Tai et al., 2002). Its expression decreases in the chorionic trophoblast of women in labor (Sangha et al., 1994; Van Meir et al., 1997). 15-HPGD hypomorphic mouse litters deliver about one day early (Roizen et al., 2008). Although progesterone withdrawal is not observed,

the levels of $\text{PGF}_{2\alpha}$ and PGE_2 rise early in these females, demonstrating the importance of a non-luteolytic role of prostaglandins in inducing labor (Roizen et al., 2008). The 15-HPGD hypomorphic mice provide an interesting genetic model for spontaneous preterm birth, and highlight the limited physiological changes that shift parturition from progesterone withdrawal to a mechanism that does not require progesterone withdrawal.

The study of genetically altered mice indicates conserved gene expression changes in the uterus at term, which display some functional redundancy. The expression of contractile-associated proteins such as FP, oxytocin receptor (Oxt_r) and connexin43 (Cx43) increase in the myometrium of both humans and mice at term (Garfield et al., 1977; Fuchs et al., 1982; Brodt-Eppley and Myatt, 1999; Cook et al., 2000). Oxytocin is a strong uterine contractile agonist and expression of its receptor (Oxt_r) increases tenfold in the uterus at term (Challis and Lye, 1994; Gainer and Wray, 1994; Zingg et al., 1995). Surprisingly, both oxytocin-deficient and Oxt_r-deficient mice exhibit normal parturition (Nishimori et al., 1996; Young et al., 1996; Takayanagi et al., 2005). By contrast, mice that are deficient in both oxytocin and cyclooxygenase-1 undergo prolonged parturition that begins at normal term (Gross et al., 1998). This suggests that oxytocin has a luteotrophic role that opposes the luteolytic role of cyclooxygenase-1, which results in normal progesterone withdrawal in these double knockout animals (Gross et al., 1998). However, cyclooxygenase-1 deficiency affects the presence of the contractile agonist $\text{PGF}_{2\alpha}$ (Gross et al., 1998). Deficiencies in both oxytocin

This systemic immune activation resembling sepsis leads to progesterone withdrawal and induction of uterine contractility. As progesterone withdrawal occurs in this model, its extension to human infection-associated preterm birth remains limited. More recently, the intrauterine application of heat-killed *Escherichia coli* (HKE) to mimic local inflammation has been evaluated (Hirsch and Muhle, 2002). In this model, preterm labor reliably ensues, but the mechanism appears distinct from progesterone withdrawal increasing its relevance to human preterm birth. Using this paradigm, essential roles for TLR4 and its downstream adapter molecule MyD88 have been demonstrated in HKE-induced preterm birth (Wang and Hirsch, 2003; Filipovich et al., 2009). By contrast, redundant signaling by the cytokines tumor necrosis factor- α (TNF- α) and interleukin-1 (IL-1) maintains susceptibility to preterm birth in mice, with the signaling of each of these cytokines being individually compromised (Hirsch et al., 2006).

Two novel pathways regulating birth timing in mice were recently described. Premature elevation of surfactant protein-A, a putative activator of macrophage proinflammatory cascades and fetal lung maturation signals, in the amniotic fluid leads to early birth (Condon et al., 2004). Similar changes in human infants in surfactant protein-A in the amniotic fluid, however, have not been found. In addition, uterine-specific disruption of p53 results in early uterine senescence and an increased frequency of preterm birth in mice (Hirota et al., 2010). Whether p53 mutations in humans are associated with preterm birth risk remains to be analyzed.

Other models of parturition

Although the sheep and mouse models are the most common, there are other valuable models for human parturition. Rats, guinea pigs, dogs and primates have all contributed to our understanding of preterm birth. The guinea pig offers a tractable small animal system, which lends itself to useful comparative genomic and physiological analysis (Mitchell and Taggart, 2009). Guinea pigs maintain high maternal serum progesterone concentrations at the time of parturition, and might be used to identify key regulatory pathways that differ in relation to closely related species that undergo progesterone withdrawal. Technical considera-

tions prevent the widespread use of guinea pigs as a parturition model. Unlike other small mammalian model systems, such as mice, a fully sequenced guinea pig genome is not yet available. Furthermore, comparatively fewer experimental protocols and reagents have been adapted for this species. However, some of the physiological similarities between guinea pig and human gestation may increase its use in the future.

Human parturition

In humans, in contrast to sheep and other ruminants, the HPA axis of the fetus does not appear to play the pivotal role in determining birth timing. In cases of human pregnancies with anencephalic fetuses (fetuses lacking pituitary and adrenal glands), gestation lengths are normal after corrections for variables such as polyhydramnios in the populations studied (Swaab et al., 1977). However, the fetal maturational effects of glucocorticoids found in animal studies (Liggins, 1968; Muglia et al., 1995) extend to humans, and glucocorticoids are commonly used to accelerate fetal lung maturation in women who are likely to enter labor preterm.

Although mice and sheep experience a dramatic decline in circulating maternal progesterone before the induction of myometrial contractility, like the predominance of mammals, such a progesterone withdrawal is not observed in term women. This lack of overt progesterone withdrawal at the end of human pregnancy has made the labor-induction pathways that are downstream of progesterone withdrawal, identified in animal models, of uncertain relevance to human pregnancy. A 'functional' withdrawal of progesterone has been hypothesized to occur at the onset of human labor (Mendelson, 2009), thus maintaining the centrality of progesterone action for birth timing. Several potential mechanisms for such a functional withdrawal in humans have been analyzed, although they have not yet been established as the key event for parturition.

The role of progesterone is known to change during human gestation owing to the presence of the progesterone receptor isoforms PR-A and PR-B, whose relative levels fluctuate during pregnancy and birth. The receptors have unique functions. PR-B induces progesterone-responsive genes. PR-A represses the transcriptional activity of PR-B. It is still difficult to identify specific

Clinical terms

Bicornuate uterus – uterus with two distinct horns, such as occurs in rodents

Cortisol – glucocorticoid hormone produced by the adrenal gland in response to ACTH production. It promotes development of the fetal lungs and other organs before birth. Before the onset of labor in sheep, rising cortisol levels promote a shift from pregnancy maintenance (via progesterone) to parturition (via estrogen)

HPA axis – hypothalamic-pituitary-adrenal axis. A highly conserved part of the neuroendocrine system that controls stress responses and the hormone changes associated with pregnancy and labor in some mammals

Luteal-placental shift – the shift from the corpus luteum to the placenta as the origin of the progesterone synthesis needed to support pregnancy

Pregnenolone – a steroid prohormone that gives rise to progesterone and estrogens as well as mineralocorticoids, glucocorticoids and androgens

Pyriiform uterus – formed when the two müllerian ducts completely fuse to form a single organ. It is normal in humans and other primates

isoforms, and little is known about the relative expression of these progesterone receptor isoforms in laboring and non-laboring human myometrium. One promising study identified an increase in the PR-A:PR-B ratio, at the protein level, in the laboring human myometrium using two isoform-specific antibodies (Merlino et al., 2007). An increase in the PR-A:PR-B ratio is also observed in the uterus of laboring rhesus macaques (Haluska et al., 2002). As with humans, rhesus macaques do not experience a decline in serum progesterone levels at term, so progesterone may be functionally withdrawn by a change in progesterone receptor isoform expression in primates.

Coactivators may also regulate functional progesterone levels in humans. Decreased expression of coactivators with acetylase activity is observed in the uterus of humans and mice at term (Condon et al., 2003). The administration of histone deacetylase inhibitor to mice during late gestation delayed the start of parturition (Condon et al., 2003). These data suggest that a decrease in coactivators and acetylation at term may impair the transcriptional activity of the progesterone-progesterone receptor complex, and implement a functional progesterone withdrawal (Condon et al., 2003).

These findings suggest that regulation of progesterone action may be a crucial step in precipitating parturition in humans. Accordingly, progesterone receptor antagonists precipitate labor in women in some cases (Herrmann et al., 1982). In addition to promoting uterine contractility, administration of progesterone receptor antagonist may increase cervical ripening in pregnant women (Radestad et al., 1993). The upregulation of contractile-associated proteins is an important component of labor induction in humans as in other species. The levels of Oxt_r, Cx43 and FP are increased in the human myometrium at term (Garfield et al., 1977; Fuchs et al., 1982; Brodt-Eppley and Myatt, 1999). Accordingly, oxytocin is commonly used to induce labor in women (Challis and Lye, 1994; Gainer and Wray, 1994). Furthermore, 15-HPGD is decreased in the chorionic trophoblast cells in laboring women (Sangha et al., 1994; Van Meir et al., 1997), contributing to increasing levels of prostaglandins, which are another important contractile agonist. Although these final common mediators that are involved in enhancing uterine contractility and cervical ripening appear to be conserved across species, their functional redundancy has limited their utility as therapeutic targets. Currently, no effective pharmacological agents have been developed to prevent prematurity, other than perhaps progesterone supplementation in some high-risk cases (Iams et al., 2008). Understanding the timing mechanisms that initiate the induction of these final common mediators, which are less clearly conserved across mammals, remains elusive.

New opportunities for progress: capitalizing on interspecies differences

The regulation of progesterone production and its decline at the end of pregnancy differs between ruminants, rodents and apes. Indeed, even amongst primate species, progesterone profiles diverge during pregnancy (Faiman et al., 1981). In humans and great apes, maternal serum progesterone concentrations rise through gestation and are at their highest at the time of delivery (Fig. 2). By contrast, Old World monkeys, such as the macaque and baboon, have much lower concentrations of circulating progesterone that do not rise through gestation, and New World monkeys display

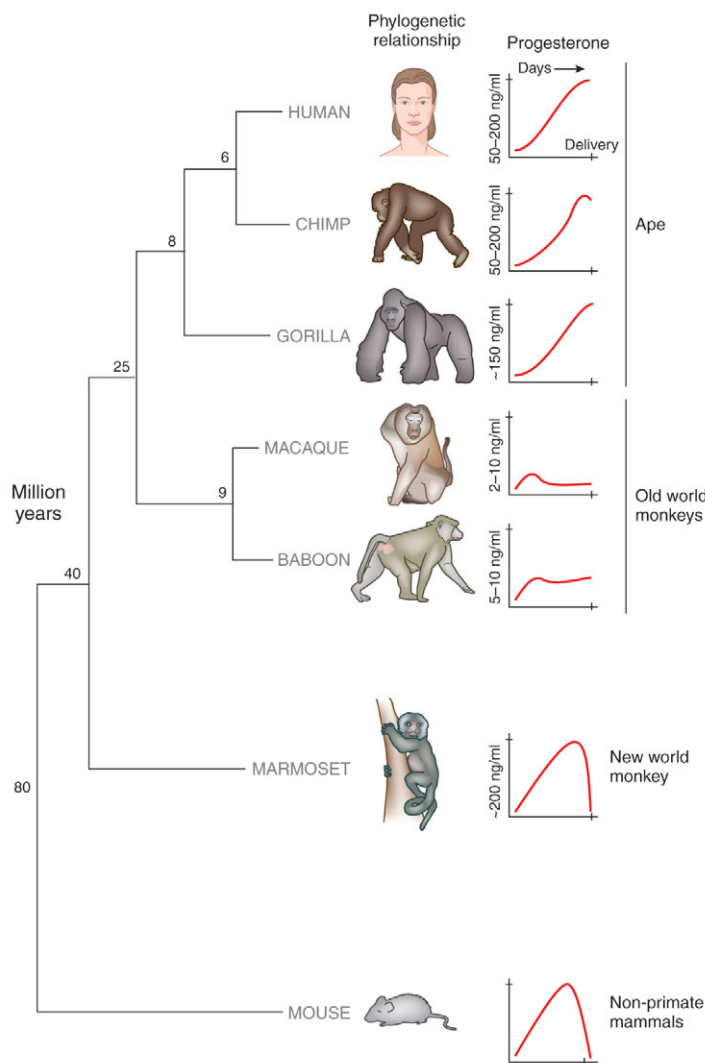


Fig. 2. Patterns of regulation of serum progesterone in humans, non-human primates and mice. Phylogenetic relationships with estimated divergence times are based upon Goodman et al. (1998). Primate serum progesterone profiles are from Reyes et al. (1975); Albrecht and Townsley (1978); Chambers and Hearn (1979); Faiman et al. (1981); Stanczyk et al. (1986); and Smith et al. (1999). Non-primate mammals, in general, show progesterone withdrawal. The guinea pig is one exception to this pattern.

overt progesterone withdrawal (Fig. 2). Consistent with these differences in progesterone regulation between mammals, are the morphological differences found in the placenta across phyla. The human placenta is characterized by a hemochorial placental interface (one in which the maternal

blood comes in direct contact with the chorion), discoidal shape and villous fetomaternal interdigitation. By using phylogenetic and statistical analyses of molecular and morphological information, Wildman and colleagues demonstrated that the ancestral eutherian mammalian placenta was

Human PATQ**R**VLSPLMSRSGCK**V**GDSSGTA**A**AHKVLP**R**GLSP**A**RQ**L**LLP**A**SE**S**PHW**S**GAPVK**P**SPQ**A**AAV
 Chimpanzee PATQ**G**VLSPLMSRSGCK**A**GDSSGTA**A**AHKVLP**R**GLSP**S**RQ**L**LLP**A**SG**S**PHC**S**GAPVK**P**SPQ**P**AAV

Fig. 3. Adaptive evolution of the progesterone receptor since the divergence of the human-chimpanzee common ancestor. The amino acids spanning the junction between the activating function-3 and inhibitory function regions in the progesterone receptor are shown. Several amino acid changes are found in this limited coding region (red) (Chen et al., 2008).

Disease Models & Mechanisms • DMM

Table 1. Relevance of common animal models for studying human parturition

	Sheep	Mice	Guinea pigs
Key advantages for use in exploring human parturition	Large mammalian system allows more facile surgical manipulation and acquisition of blood/amniotic fluid measures	Genetic tractability, short gestation	Initiation of parturition without progesterone withdrawal like in humans
Key limitations for use in exploring human parturition	Progesterone withdrawal at parturition onset	Progesterone withdrawal at parturition onset	Lack of sequenced genome; less-developed methods and reagents for experimentation than other small mammalian model systems
Potential areas with applicability to human parturition	Regulation of fetal maturation, control of myometrial contractility	Contribution of specific genes to uterine contractility, cervical ripening and inflammation; exploration of the effects of gene isoforms and variants identified through computational biology approaches	Parturition initiation, mechanisms for labor induction without progesterone withdrawal

itself hemochorial and discoid (Wildman et al., 2006). Thus, surprisingly, the human placenta is a primitive state that emerged before the origin of primates. These findings suggest that the major roles of the placenta in sustaining pregnancy and facilitating fetal growth have persisted throughout the eutherian lineage that has led to humans.

One inference emerging from the observed divergence in endocrine physiology and placental morphology between species, is that evolutionary pressures have uniquely optimized the timing and characteristics of pregnancy (number of fetuses, uterine architecture, sites of hormone production, etc.) to enhance the reproductive success and minimize maternal and fetal mortality. In accord with this notion, sequence comparison of human, chimpanzee and mouse genomes revealed that the genes associated with reproduction are one of the most divergent functional categories across these species (Chimpanzee Sequencing and Analysis Consortium, 2005). Are these differences instructional? Using currently available computational biology approaches, together with a growing list of mammalian genomes sequenced at high coverage, the potential exists to identify rapidly diverging genes. Of particular relevance to human-specific parturition biology may be genes that have evolved rapidly along the human lineage. Rapidly evolving genes would be plausible candidates for being involved in human parturition and would not require conformation to present notions about human pregnancy physiology. The progesterone receptor itself exhibits characteristics of rapid adaptive

evolution in humans (Fig. 3) (Chen et al., 2008). The intersection of the emerging list of rapidly evolving genes and conserved noncoding sequences (Bustamante et al., 2006; Prabhakar et al., 2006; Pollard et al., 2006) could narrow the spectrum of variants considered by genome-wide association studies, and provide new targets for manipulation in mice and other genetically tractable organisms. By introducing these specific human variants or isoforms (for example, in progesterone receptor A or B) with temporally and spatially regulated transgenic expression systems in mice or other model systems, their ability to affect birth timing could be investigated systematically.

Although animal models are not without their limitations in modeling human parturition biology, they have made important contributions to our current understanding of this complex process. Differences in how parturition is executed in common animal models and humans have been identified, but these differences should not discourage investigators from conducting further research to identify additional important similarities. Specific examples of the aspects of parturition in different animal models are summarized in Table 1. The list in this table is not all encompassing, but highlights areas that are analogous, or discordant, in human pregnancy. Furthermore, combining the established and versatile mouse model system with the computation biology approach outlined here points to an exciting new frontier in the study of human parturition through animal models.

COMPETING INTERESTS

The authors declare no competing financial interests.

Clinical and basic research opportunities

- Identify the evolutionary changes that influence preterm birth
- Determine the physiological changes associated with infection that encourage parturition
- Create new animal models to test the influence of genes suggested by human association studies on parturition
- Develop a physiological model to test theories of 'functional' progesterone withdrawal in the induction of human labor

REFERENCES

Adams, M. M., Elam-Evans, L. D., Wilson, H. G. and Gilbertz, D. A. (2000). Rates of and factors associated with recurrence of preterm delivery. *Jama* **283**, 1591-1596.

Ahman, E. and Zupan, J. (2007). *Neonatal and Perinatal Mortality: Country, Regional, and Global Estimates 2004*. Geneva: World Health Organization.

Albrecht, E. D. and Townsley, J. D. (1978). Serum estradiol in mid and late gestation and estradiol/progesterone ratio in baboons near parturition. *Biol. Reprod.* **18**, 247-250.

Bethin, K. E., Nagai, Y., Sladek, R., Asada, M., Sadovsky, Y., Hudson, T. J. and Muglia, L. J. (2003). Microarray analysis of uterine gene expression in mouse and human pregnancy. *Mol. Endocrinol.* **17**, 1454-1469.

Bonventre, J., Huang, Z., Taheri, M., O'Leary, E., Li, E., Moskowitz, M. and Sapirstein, A. (1997). Reduced fertility and postischemic brain injury in mice deficient in cytosolic phospholipase A2. *Nature* **390**, 622-625.

Brodth-Eppley, J. and Myatt, L. (1999). Prostaglandin receptors in lower segment myometrium during gestation and labor. *Obstet. Gynecol.* **93**, 89-93.

Bustamante, C. D., Fedel-Alon, A., Williamson, S., Nielsen, R., Hubisz, M. T., Glanowski, S., Tanenbaum, D. M., White, T. J., Sninsky, J. J., Hernandez, R. D. et al. (2005). Natural selection on protein-coding genes in the human genome. *Nature* **437**, 1153-1157.

Challis, J. and Lye, S. (1994). *The Physiology of Reproduction*. New York: Raven.

- Challis, J. R. G., Matthews, S. G., Gibb, W. and Lye, S. J.** (2000). Endocrine and paracrine regulation of birth at term and preterm. *Endocr. Rev.* **21**, 514-550.
- Chambers, P. L. and Hearn, J. P.** (1979). Peripheral plasma levels of progesterone, oestradiol-17 beta, oestrone, testosterone, androstenedione and chorionic gonadotrophin during pregnancy in the marmoset monkey, *Callithrix jacchus*. *J. Reprod. Fertil.* **56**, 23-32.
- Chen, C., Opazo, J. C., Erez, O., Uddin, M., Santolaya-Forgas, J., Goodman, M., Grossman, L. I., Romero, R. and Wildman, D. E.** (2008). The human progesterone receptor shows evidence of adaptive evolution associated with its ability to act as a transcription factor. *Mol. Phylogenet. Evol.* **47**, 637-649.
- Chimpanzee Sequencing and Analysis Consortium** (2005). Initial sequence of the chimpanzee genome and comparison with the human genome. *Nature* **437**, 69-87.
- Committee on Understanding Premature Birth and Assuring Healthy Outcomes Board on Health Sciences Policy** (2006). *Preterm Birth: Causes, Consequences, and Prevention*. Washington, DC: The National Academies Press.
- Condon, J., Jeyasuria, P., Faust, J., Wilson, J. and Mendelson, C.** (2003). 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* **100**, 9518-9523.
- Condon, J., Jeyasuria, P., Faust, J. and Mendelson, C.** (2004). Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. *Proc. Natl. Acad. Sci. USA* **101**, 4978-4983.
- Cook, J., Zaragoza, D., Sung, D. and Olson, D.** (2000). Expression of myometrial activation and stimulation genes in a mouse model of preterm labor: myometrial activation, stimulation, and preterm labor. *Endocrinology* **141**, 1718-1728.
- Crider, K. S., Whitehead, N. and Buus, R. M.** (2005). Genetic variation associated with preterm birth: a HuGE review. *Genet. Med.* **7**, 593-604.
- Doring, B., Shynlova, O., Tsui, P., Eckardt, D., Janssen-Bienhold, U., Hofmann, F., Feil, S., Feil, R., Lye, S. J. and Willecke, K.** (2006). Ablation of connexin43 in uterine smooth muscle cells of the mouse causes delayed parturition. *J. Cell Sci.* **119**, 1715-1722.
- Faiman, C., Reyes, F. I., Winter, J. S. D. and Hobson, W. C.** (1981). Endocrinology of pregnancy in apes. In *Reproductive Biology of the Great Apes: Comparative and Biomedical Perspectives* (ed. C. E. Graham), pp. 45-68. London: Academic Press.
- Fidel, P., Jr, Romero, R., Maymon, E. and Hertelendy, F.** (1998). Bacteria-induced or bacterial product-induced preterm parturition in mice and rabbits is preceded by a significant fall in serum progesterone concentrations. *J. Matern. Fetal. Med.* **7**, 222-226.
- Filipovich, Y., Lu, S. J., Akira, S. and Hirsch, E.** (2009). The adaptor protein MyD88 is essential for E coli-induced preterm delivery in mice. *Am. J. Obstet. Gynecol.* **200**, 93 e91-e98.
- Fuchs, A., Fuchs, F., Husslein, P., Soloff, M. and Fernstrom, M.** (1982). Oxytocin receptors and human parturition: a dual role for oxytocin in the initiation of labor. *Science* **215**, 1396-1398.
- Gainer, H. and Wray, S.** (1994). *The Physiology of Reproduction*. New York: Raven.
- Garfield, R., Sims, S. and Daniel, E.** (1977). Gap junctions: their presence and necessity in myometrium during parturition. *Science* **198**, 958-960.
- Goldenberg, R. L., Cliver, S. P., Mulvihill, F. X., Hickey, C. A., Hoffman, H. J., Klerman, L. V. and Johnson, M. J.** (1996). Medical, psychosocial, and behavioral risk factors do not explain the increased risk for low birth weight among black women. *Am. J. Obstet. Gynecol.* **175**, 1317-1324.
- Goodman, M., Porter, C. A., Czelusniak, J., Page, S. L., Schneider, H., Shoshani, J., Gunnell, G. and Groves, C. P.** (1998). Toward a phylogenetic classification of Primates based on DNA evidence complemented by fossil evidence. *Mol. Phylogenet. Evol.* **9**, 585-598.
- Gross, G. A., Imamura, T., Luedke, C., Vogt, S. K., Olson, L. M., Nelson, D. M., Sadovsky, Y. and Muglia, L. J.** (1998). Opposing actions of prostaglandins and oxytocin determine the onset of murine labor. *Proc. Natl. Acad. Sci. USA* **95**, 11875-11879.
- Gross, G., Imamura, T., Vogt, S., Wozniak, D., Nelson, D., Sadovsky, Y. and Muglia, L.** (2000). Inhibition of cyclooxygenase-2 prevents inflammation-mediated preterm labor in the mouse. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **278**, R1415-R1423.
- Haluska, G. J., Wells, T. R., Hirst, J. J., Brenner, R. M., Sadovsky, D. W. and Novy, M. J.** (2002). Progesterone receptor localization and isoforms in myometrium, decidua, and fetal membranes from rhesus macaques: evidence for functional progesterone withdrawal at parturition. *J. Soc. Gynecol. Investig.* **9**, 125-136.
- Heron, M., Sutton, P. D., Xu, J., Ventura, S. J., Strobino, D. M. and Guyer, B.** (2010). Annual summary of vital statistics: 2007. *Pediatrics* **125**, 4-15.
- Herrmann, W., Wyss, R., Riondel, A., Philibert, D., Teutsch, G., Sakiz, E. and Baulieu, E.** (1982). The effects of an antiprogesterone steroid in women: interruption of the menstrual cycle and of early pregnancy. *C. R. Acad. Sci. Ser. III* **294**, 933-938.
- Hirota, Y., Daikoku, T., Tranguch, S., Xie, H., Bradshaw, H. B. and Dey, S. K.** (2010). Uterine-specific p53 deficiency confers premature uterine senescence and promotes preterm birth in mice. *J. Clin. Invest.* **120**, 803-815.
- Hirsch, E. and Muhle, R.** (2002). Intrauterine bacterial inoculation induces labor in the mouse by mechanisms other than progesterone withdrawal. *Biol. Reprod.* **67**, 1337-1341.
- Hirsch, E., Filipovich, Y. and Mahendroo, M.** (2006). Signaling via the type I IL-1 and TNF receptors is necessary for bacterially induced preterm labor in a murine model. *Am. J. Obstet. Gynecol.* **194**, 1334-1340.
- Iams, J. D., Romero, R., Culhane, J. F. and Goldenberg, R. L.** (2008). Primary, secondary, and tertiary interventions to reduce the morbidity and mortality of preterm birth. *Lancet* **371**, 164-175.
- Kistka, Z. A., Palomar, L., Lee, K. A., Boslaugh, S. E., Wangler, M. F., Cole, F. S., DeBaun, M. R. and Muglia, L. J.** (2007). Racial disparity in the frequency of recurrence of preterm birth. *Am. J. Obstet. Gynecol.* **196**, 131 e131-e136.
- Kistka, Z. A., DeFranco, E. A., Ligthart, L., Willemsen, G., Plunkett, J., Muglia, L. J. and Boomsma, D. I.** (2008). Heritability of parturition timing: an extended twin design analysis. *Am. J. Obstet. Gynecol.* **199**, 43 e41-e45.
- Kitts, D. D., Anderson, G. B., BonDurant, R. H. and Stabenfeldt, G. H.** (1984). Temporal patterns of delta 4 C-21 steroids in coexisting, genetically dissimilar twin lamb fetuses throughout late gestation. *Endocrinology* **114**, 703-711.
- Krajnc-Franken, M. A., van Disseldorp, A. J., Koenders, J. E., Mosselman, S., van Duin, M. and Gossen, J. A.** (2004). Impaired nipple development and parturition in LGR7 knockout mice. *Mol. Cell. Biol.* **24**, 687-696.
- Langenbach, R., Morham, S. G., Tian, H. F., Loftin, C. D., Ghanayem, B. I., Chulada, P. C., Mahler, J. F., Lee, C. A., Goulding, E. H., Kluckman, K. D. et al.** (1995). Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell* **83**, 483-492.
- Leppert, P. C.** (1995). Anatomy and physiology of cervical ripening. *Clin. Obstet. Gynecol.* **38**, 267-279.
- Liggins, G. C.** (1968). Premature parturition after infusion of corticotrophin or cortisol into foetal lambs. *J. Endocrinol.* **42**, 323-329.
- Liggins, G. C., Kennedy, P. C. and Holm, L. W.** (1967). Failure of initiation of parturition after electrocoagulation of the pituitary of the fetal lamb. *Am. J. Obstet. Gynecol.* **98**, 1080-1086.
- Mahendroo, M. S., Cala, K. M. and Russell, D. W.** (1996). 5 alpha-reduced androgens play a key role in murine parturition. *Mol. Endocrinol.* **10**, 380-392.
- Mahendroo, M. S., Porter, A., Russell, D. W. and Word, R. A.** (1999). The parturition defect in steroid 5alpha-reductase type 1 knockout mice is due to impaired cervical ripening. *Mol. Endocrinol.* **13**, 981-992.
- Martin, J. A., Hamilton, B. E., Sutton, P. D., Ventura, S. J., Menacker, F., Kirmeyer, S. and Mathews, F. S.** (2009). Births: final data for 2006. *Natl. Vital Stat. Rep.* **57**, 1-102.
- Mendelson, C. R.** (2009). Minireview: fetal-maternal hormonal signaling in pregnancy and labor. *Mol. Endocrinol.* **23**, 947-954.
- Merlino, A. A., Welsh, T. N., Tan, H., Yi, L. J., Cannon, V., Mercer, B. M. and Mesiano, S.** (2007). Nuclear progesterone receptors in the human pregnancy myometrium: evidence that parturition involves functional progesterone withdrawal mediated by increased expression of progesterone receptor-A. *J. Clin. Endocrinol. Metab.* **92**, 1927-1933.
- Mitchell, B. F. and Taggart, M. J.** (2009). Are animal models relevant to key aspects of human parturition? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **297**, R525-R545.
- Muglia, L. J. and Katz, M.** (2010). The enigma of spontaneous preterm birth. *N. Engl. J. Med.* **362**, 529-535.
- Muglia, L., Jacobson, L., Dikkes, P. and Majzoub, J. A.** (1995). Corticotropin-releasing hormone deficiency reveals major fetal but not adult glucocorticoid need. *Nature* **373**, 427-432.
- Murray, C. J. and Lopez, A. D.** (1997). Global mortality, disability, and the contribution of risk factors: global burden of disease study. *Lancet* **349**, 1436-1442.
- Nishimori, K., Young, L. J., Guo, Q., Wang, Z., Insel, T. R. and Matzuk, M. M.** (1996). Oxytocin is required for nursing but is not essential for parturition or reproductive behavior. *Proc. Natl. Acad. Sci. USA* **93**, 11699-11704.
- Okita, R. T. and Okita, J. R.** (1996). Prostaglandin-metabolizing enzymes during pregnancy: characterization of NAD(+)-dependent prostaglandin dehydrogenase, carbonyl reductase, and cytochrome P450-dependent prostaglandin omega-hydroxylase. *Crit. Rev. Biochem. Mol. Biol.* **31**, 101-126.
- Onderdonk, A. B., Delaney, M. L., DuBois, A. M., Allred, E. N. and Leviton, A.** (2008). Detection of bacteria in placental tissues obtained from extremely low gestational age neonates. *Am. J. Obstet. Gynecol.* **198**, 110 e111-e117.
- Palomar, L., DeFranco, E. A., Lee, K. A., Allsworth, J. E. and Muglia, L. J.** (2007). Paternal race is a risk factor for preterm birth. *Am. J. Obstet. Gynecol.* **197**, 152 e151-e157.
- Piekorz, R. P., Gingras, S., Hoffmeyer, A., Ihle, J. N. and Weinstein, Y.** (2005). Regulation of progesterone levels during pregnancy and parturition by signal transducer and activator of transcription 5 and 20alpha-hydroxysteroid dehydrogenase. *Mol. Endocrinol.* **19**, 431-440.

- Plunkett, J. and Muglia, L. J.** (2008). Genetic contributions to preterm birth: implications from epidemiological and genetic association studies. *Ann. Med.* **40**, 167-195.
- Plunkett, J., Feitosa, M. F., Trusgnich, M., Wangler, M. F., Palomar, L., Kistka, Z. A., DeFranco, E. A., Shen, T. T., Stormo, A. E. D., Puttonen, H. et al.** (2009). Mother's genome or maternally-inherited genes acting in the fetus influence gestational age in familial preterm birth. *Hum. Hered.* **68**, 209-219.
- Pollard, K. S., Salama, S. R., King, B., Kern, A. D., Dreszer, T., Katzman, S., Siepel, A., Pedersen, J. S., Bejerano, G., Baertsch, R. et al.** (2006). Forces shaping the fastest evolving regions in the human genome. *PLoS Genet.* **2**, e168.
- Porter, T. F., Fraser, A. M., Hunter, C. Y., Ward, R. H. and Varner, M. W.** (1997). The risk of preterm birth across generations. *Obstet. Gynecol.* **90**, 63-67.
- Prabhakar, S., Noonan, J. P., Paabo, S. and Rubin, E. M.** (2006). Accelerated evolution of conserved noncoding sequences in humans. *Science* **314**, 786.
- Radestad, A., Thyberg, J. and Christensen, N. J.** (1993). Cervical ripening with mifepristone (RU 486) in first trimester abortion. An electron microscope study. *Hum. Reprod.* **8**, 1136-1142.
- Ratajczak, C. K. and Muglia, L. J.** (2008). Insights into parturition biology from genetically altered mice. *Pediatr. Res.* **64**, 581-589.
- Reaume, A., de Sousa, P., Kulkarni, S., Langille, B., Zhu, D., Davies, T., Juneja, S., Kidder, G. and Rossant, J.** (1995). Cardiac malformation in neonatal mice lacking connexin43. *Science* **267**, 1831-1834.
- Reyes, F. I., Winter, J. S., Faiman, C. and Hobson, W. C.** (1975). Serial serum levels of gonadotropins, prolactin and sex steroids in the nonpregnant and pregnant chimpanzee. *Endocrinology* **96**, 1447-1455.
- Roizen, J. D., Asada, M., Tong, M., Tai, H. H. and Muglia, L. J.** (2008). Preterm birth without progesterone withdrawal in 15-hydroxyprostaglandin dehydrogenase hypomorphic mice. *Mol. Endocrinol.* **22**, 105-112.
- Sangha, R., Walton, J., Ensor, C., Tai, H. and Challis, J.** (1994). Immunohistochemical localization, messenger ribonucleic acid abundance, and activity of 15-hydroxyprostaglandin dehydrogenase in placenta and fetal membranes during term and preterm labor. *J. Clin. Endocrinol. Metab.* **78**, 982-989.
- Skarnes, R. C. and Harper, M. J.** (1972). Relationship between endotoxin-induced abortion and the synthesis of prostaglandin F. *Prostaglandins* **1**, 191-203.
- Smith, R., Wickings, E. J., Bowman, M. E., Belleoud, A., Dubreuil, G., Davies, J. J. and Madsen, G.** (1999). Corticotropin-releasing hormone in chimpanzee and gorilla pregnancies. *J. Clin. Endocrinol. Metab.* **84**, 2820-2825.
- Stanczyk, F. Z., Hess, D. L., Namkung, P. C., Senner, J. W., Petra, P. H. and Novy, M. J.** (1986). Alterations in sex steroid-binding protein (SBP), corticosteroid-binding globulin (CBG), and steroid hormone concentrations during pregnancy in rhesus macaques. *Biol. Reprod.* **35**, 126-132.
- Sugimoto, Y., Yamasaki, A., Segi, E., Tsuboi, K., Aze, Y., Nishimura, T., Oida, H., Yoshida, N., Tanaka, T., Katsuyama, M. et al.** (1997). Failure of parturition in mice lacking the prostaglandin F receptor. *Science* **277**, 681-683.
- Swaab, D. F., Boer, K. and Honnebier, W. J.** (1977). The influence of the fetal hypothalamus and pituitary on the onset and course of parturition. *Ciba Found. Symp.* **47**, 379-400.
- Tai, H. H., Ensor, C. M., Tong, M., Zhou, H. and Yan, F.** (2002). Prostaglandin catabolizing enzymes. *Prostaglandins Other Lipid Mediat.* **68-69**, 483-493.
- Takayanagi, Y., Yoshida, M., Bielsky, I. F., Ross, H. E., Kawamata, M., Onaka, T., Yanagisawa, T., Kimura, T., Matzuk, M. M., Young, L. J. et al.** (2005). Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. *Proc. Natl. Acad. Sci. USA* **102**, 16096-16101.
- Uozumi, N., Kume, K., Nagase, T., Nakatani, N., Ishii, S., Tashiro, F., Komagata, Y., Maki, K., Ikuta, K., Ouchi, Y. et al.** (1997). Role of cytosolic phospholipase A2 in allergic response and parturition. *Nature* **390**, 618-621.
- Van Meir, C., Ramirez, M., Matthews, S., Calder, A., Keirse, M. and Challis, J.** (1997). Chorionic prostaglandin catabolism is decreased in the lower uterine segment with term labour. *Placenta* **18**, 109-114.
- Wang, H. and Hirsch, E.** (2003). Bacterially-induced preterm labor and regulation of prostaglandin-metabolizing enzyme expression in mice: the role of toll-like receptor 4. *Biol. Reprod.* **69**, 1957-1963.
- Wang, H., Parry, S., Macones, G., Sammel, M. D., Kuivaniemi, H., Tromp, G., Argyropoulos, G., Halder, I., Shriver, M. D., Romero, R. et al.** (2006). A functional SNP in the promoter of the SERPINH1 gene increases risk of preterm premature rupture of membranes in African Americans. *Proc. Natl. Acad. Sci. USA* **103**, 13463-13467.
- Watts, D. H., Krohn, M. A., Hillier, S. L. and Eschenbach, D. A.** (1992). The association of occult amniotic fluid infection with gestational age and neonatal outcome among women in preterm labor. *Obstet. Gynecol.* **79**, 351-357.
- Wiest, W.** (1968). On the function of 20 alpha-hydroxypren-4-en-3-one during parturition in the rat. *Endocrinology* **83**, 1181-1184.
- Wildman, D. E., Chen, C., Erez, O., Grossman, L. I., Goodman, M. and Romero, R.** (2006). Evolution of the mammalian placenta revealed by phylogenetic analysis. *Proc. Natl. Acad. Sci. USA* **103**, 3203-3208.
- Winkvist, A., Mogren, I. and Hogberg, U.** (1998). Familial patterns in birth characteristics: impact on individual and population risks. *Int. J. Epidemiol.* **27**, 248-254.
- Young, W. S., 3rd, Shepard, E., Amico, J., Hennighausen, L., Wagner, K. U., LaMarca, M. E., McKinney, C. and Ginns, E. I.** (1996). Deficiency in mouse oxytocin prevents milk ejection, but not fertility or parturition. *J. Neuroendocrinol.* **8**, 847-853.
- Zhao, L., Roche, P. J., Gunnarsen, J. M., Hammond, V. E., Tregear, G. W., Wintour, E. M. and Beck, F.** (1999). Mice without a functional relaxin gene are unable to deliver milk to their pups. *Endocrinology* **140**, 445-453.
- Zhao, L., Samuel, C. S., Tregear, G. W., Beck, F. and Wintour, E. M.** (2000). Collagen studies in late pregnant relaxin null mice. *Biol. Reprod.* **63**, 697-703.
- Zingg, H. H., Rozen, F., Chu, K., Larcher, A., Arslan, A., Richard, S. and Lefebvre, D.** (1995). Oxytocin and oxytocin receptor gene expression in the uterus. *Recent Prog. Horm. Res.* **50**, 255-273.