Are animal models relevant to key aspects of human parturition?

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Mitchell BF, Taggart MJ. Are animal models relevant to key aspects of human parturition? Am J Physiol Regul Integr Comp Physiol 297: R525–R545, 2009. First published June 10, 2009; doi:10.1152/ajpregu.00153.2009.—Preterm birth remains the most serious complication of pregnancy and is associated with increased rates of infant death or permanent neurodevelopmental disability. Our understanding of the regulation of parturition remains inadequate. The scientific literature, largely derived from rodent animal models, suggests two major mechanisms regulating the timing of parturition: the withdrawal of the steroid hormone progesterone and a proinflammatory response by the immune system. However, available evidence strongly suggests that parturition in the human has significantly different regulators and mediators from those in most of the animal models. Our objectives are to critically review the data and concepts that have arisen from use of animal models for parturition and to rationalize the use of a new model. Many animal models have contributed to advances in our understanding of the regulation of parturition. However, we suggest that those animals dependent on progesterone withdrawal to initiate parturition clearly have a limitation to their translation to the human. In such models, a linear sequence of events (e.g., luteolysis, progesterone withdrawal, uterine activation, parturition) gives rise to the concept of a “trigger” mechanism. Conversely, we propose that human parturition may arise from the concomitant maturation of several systems in parallel. We have termed this novel concept “modular accumulation of physiological systems” (MAPS). We also emphasize the urgency to determine the precise role of the immune system in the process of parturition in situations other than intrauterine infection. Finally, we accentuate the need to develop a nonprimate animal model whose physiology is more relevant to human parturition. We suggest that the guinea pig displays several key physiological characteristics of gestation that more closely resemble human pregnancy than do currently favored animal models. We conclude that the application of novel concepts and new models are required to advance translational research in parturition.

Clinical and Biological Perspectives

The immediate motivation for addressing the regulation of parturition relates to the importance of the human clinical consequences of being born too early. Although infection is a significant precedent of preterm labor, in the majority of cases there is no overt, overriding pathological cause, and the mechanism of parturition appears to represent a premature triggering of the normal mechanisms occurring at term. In all developed countries, preterm birth (≤37 wk of gestation) is associated with ~75% of newborn death or permanent disability (86, 98a). The long-term consequences that occur much more commonly in babies born preterm include both motor and cognitive neurodevelopmental disabilities that encompass cerebral palsy, blindness, deafness, mental retardation, learning disabilities, or chronic lung disease. In addition to this tremendous waste of human potential, the monetary cost to developed societies in the Western world is estimated to be in the tens of billions of dollars annually. Consideration of the financial impact must include the intensive care of the newborn in addition to the longer term costs of remedial/special educational programs, social assistance programs, institutional care, and other services for the disabled survivors and their families (17, 130, 224). Current therapeutic approaches are directed toward arrest of established preterm labor, and these have been disappointingly unsuccessful (96). Better understanding of the mechanisms that regulate the timing of parturition could lead to novel strategies for early diagnosis, treatment, or even primary prevention of preterm birth. National charitable organizations such as the March of Dimes, Action Medical Research, and the Institute of Medicine have recently recognized the dearth of knowledge in this area and have recommended programs of intense, focused research (17, 93).

The objective of this review is to provide our perspective of several important concepts that should be considered in this research thrust. We begin by presenting the scientific frame-
work, derived from animal models, that forms the basis of our current understanding of mechanisms of parturition. We then examine these findings compared with data obtained from investigations in the human. This engenders a discussion concerning the conceptual differences among species and leads to our proposals to consider new concepts and new models for future studies. We conclude by suggesting what we feel are the most important fundamental questions that need to be addressed in these studies.

In addition to the clinical perspective, there is a compelling biological rationale for enhancement of research into the physiologic of uterine function. Clearly, successful reproductive outcome is one of the most essential components of evolutionary change. Study of the marked diversities of mechanisms of parturition amongst species could provide greater understanding of the biological systems created by evolutionary forces, and it may be possible to generalize these to other physiological organ systems. For example, the study of the various paracrine and autocrine systems that function within the uterus could enhance understanding of similar processes in other organs and vice versa. Also, during normal pregnancy, considerable subcellular, cellular, and intercellular remodeling occurs in a physiological context that may present an excellent model to study adaptive mechanisms that may have broader applicability. Similarly, in relative terms, human uterine smooth muscle tissues (e.g., from hysterectomy) are abundant compared with the availability for experimentation of other uncompromised human smooth muscles. As such, uterine smooth muscle remodeling may be a good model to improve our understanding of phenotypic malleability of somewhat analogous, but less available, tissues such as human vascular smooth muscle. This also may hold true for pathophysiological remodeling of uterine tissue accompanying preterm labor and its relevance for other organ systems exposed to proinflammatory insult. As discussed throughout this article, the selection of an animal model most closely reflecting human physiology will provide the optimal information.

The Scientific Framework of Parturition

Our objective is to present an overview of the current state of knowledge regarding regulation of the onset and maintenance of human parturition. On this basis, we offer viewpoints concerning the major deficiencies in our understanding. We also suggest directions for future research, particularly the need for a better animal model of human labor and preterm labor. Our objective is not to provide an exhaustive review of any particular facet of this field of research. The bibliography contains many excellent reviews and original research articles that direct the reader to the details of specific areas.

The research data overview is provided in two sections. The first addresses the regulation of the mechanisms of birth with a focus on the endocrine/paracrine and the immune regulatory systems. The second presents data concerning the target systems that appear to be central to the process of parturition. Again, the focus is on two specific and distinct processes: the contractile mechanisms of the uterine smooth muscle and the process of cervical ripening.

Most of the information to be presented is derived from a variety of animal models. By comparing and contrasting these data from different species, we can examine the similarities and differences in the light of the relatively sparse knowledge regarding the human parturient process. This will guide our discussion of the relevance of the current animal models to the human.

Regulatory systems. Endocrine/Paracrine Mechanisms and Progesterone Withdrawal. Several animal models have been useful in examining specific molecular/cellular/tissue signaling pathways of endocrine, paracrine, or autocrine origin that may predetermine parturition onset at term or preterm. Based mainly on animal models that have a rapid withdrawal of progesterone from the maternal serum immediately before parturition, many investigators have found it helpful to consider uterine activity through pregnancy in terms of three separate phases: 1) uterine quiescence, lasting most of pregnancy, whereby the uterus remains relatively quiet and nonresponsive to stimuli; 2) the process of uterine activation, wherein the uterus is transformed from the quiescent state through modulation of the receptors and signaling pathways to respond to contractile stimuli with coordinated, forceful contractions; and 3) the stimulation phase that is characterized by active contractions and delivery of the products of conception (33, 36). In general, the data indicate that estrogen increases expression of genes associated with uterine activation and that progesterone will suppress these genes as well as stimulate genes that result in uterine relaxation. Over the next few paragraphs, we develop the proposal that these animal models do not provide an entirely relevant representation of the complexity of physiological systems that regulate uterine contractility in pregnant women.

The early studies of parturition assessed hormonal mechanisms by measuring hormone concentrations in various fluid compartments, but mainly in the maternal serum. More recently, it has become clear that the intrauterine tissues themselves have highly developed systems to produce and metabolize many classes of hormones. It is quite likely that, at least in the pregnant woman, regulation of hormone dynamics in intrauterine paracrine and autocrine systems is at least as important as are endocrine pathways.

For over 50 years progesterone has been recognized as the major progestational hormone in most animal species. Early studies (see below), principally using rabbit and rat models, gave rise to the “progesterone block” theory proposed by Csapo and others (53, 57). It was demonstrated that a withdrawal of progesterone from the maternal circulation was necessary and sufficient to cause the uterine and cervical changes eventuating in parturition (Fig. 1). Surgical or pharmacological antagonism of progesterone caused parturition, and this was prevented with progesterone supplementation, which also delayed normal parturition. According to this theory, progesterone promotes uterine quiescence by stimulation of relaxant pathways and suppression of stimulatory pathways. In the animal models used, parturition occurs only after a withdrawal of progesterone. Many subsequent investigations demonstrated that progesterone suppressed the expression of several procontractile pathways in the pregnant uterus and stimulated expression of relaxant systems. Despite a large body of experimental data, the mechanism(s) through which progesterone accomplishes its effects remain unclear. The progesterone receptor is a classic member of the nuclear receptor superfamily of ligand-activated transcription factors that interact with cognate response elements in the regulatory regions
of target genes to increase or decrease transcription. Surprisingly, many of the putative target genes of progesterone do not appear to have such response elements. Thus the mechanisms underlying the major effects of progesterone may be indirect and involve regulation of other intermediary transcription factors, coactivators/repressors, and genes. Recently, a family of plasma membrane receptors for progesterone has been reported, but the function of these in parturition has been scarcely explored (113, 192). In addition, progesterone (or its metabolite, 5β-dihydroprogesterone) has been shown to directly inhibit binding of oxytocin (OT) to its receptor (OTR), thus potentially interfering with the effects of this potent contractile agonist (92). However, the concentrations at which this effect is observed are probably beyond the physiological range and would require acceptance of the (still possible) notion of increased concentrations of steroids at cellular interfaces compared with serum.

The mechanisms underlying progesterone withdrawal for the individual animal models is discussed in more detail in a following section. However, perhaps the most disturbing facet of the progesterone block theory is that, in pregnant women, parturition occurs in the face of extremely high and unchanging or increasing maternal plasma progesterone concentrations (19, 215). Despite repeated efforts to demonstrate progesterone withdrawal, only one systematic study was able to measure a decrease in maternal progesterone concentrations before parturition (216), and this has not been confirmed. However, individual patient-to-patient variation may mask significant concentration changes in the group as a whole (205). In an attempt to rationalize these findings with the well-studied animal models, several research groups have pursued the idea of a “functional” progesterone withdrawal to cover the possibility that there could be withdrawal of the quiescent effects of progesterone despite the unchanging or increasing concentrations in maternal plasma. There are four major paradigms that could accomplish this functional progesterone withdrawal (Fig. 2).

The first concept, and the one for which most experimental evidence exists, is the “paracrine hypothesis.” Accordingly, progesterone is synthesized within a local intrauterine network, including the fetal membranes (amnion and chorion) and maternal decidua, which controls progesterone concentrations in the underlying uterine muscle (myometrium). Significant changes in progesterone production or metabolism could occur in this network, and this could affect myometrial progesterone concentrations and contractility without changes occurring in the maternal systemic circulation. Several investigators have shown that both estrogen and progesterone are produced and metabolized by human fetal membranes and decidua (81, 82, 141, 142). Moreover, it appears that, before labor, the net steroidogenic effect of this paracrine network is to produce progesterone and a weak estrogen (estrone), but, following labor onset, the predominant products are inactive progesterone metabolites and the biologically active estradiol (145). The finding of an increase in...
amniotic fluid estrogen-to-progesterone ratio at the time of parturition supports the potential clinical relevance of these data (186). In addition to steroids, this intrauterine paracrine network produces other potentially important hormones such as OT, endothelin (ET)-1, and prostaglandin F2α (PGF2α), as well as prostacyclin (PGI2) and nitric oxide (NO) (31, 41, 158, 178). Many investigators in the field now believe that paracrine interactions within the pregnant uterus are physiological regulators of uterine contractility and that, in the human, these events may be more important than the endocrine pathways that are predominant in most animal models.

A second mechanism of functional progesterone withdrawal relates to the expression of progesterone receptor (PR) isoforms. The full-length isoform, PR-B, is the major mediator of progesterone effects. However, there is an alternate transcription start site that codes for a protein, PR-A, that is identical to PR-B except that it lacks the NH2-terminal 163 amino acids that contain one of the activating functional domains (AF3) of PR-B (105). PR-A tends to have effects that oppose those of PR-B through the actions of an inhibitory function that is revealed because of the absence of AF3 (106). In both rat and human uterus, mRNA for PR-A appears to increase significantly before labor onset at term, whereas PR-B remains stable or increases only slightly (67, 140). In the human, recent evidence suggests this also is true at the level of protein (139). The possibility of an alteration of PR-A:PR-B as a means of inducing a functional progesterone withdrawal has collected considerable attention. Another putative truncated PR isoform, PR-C, that serves to inhibit PR-B function has been proposed to show increased expression before labor onset, and the possible roles of other PR splice variants remain unresolved (51, 148). In addition, as mentioned above, there is a recent suggestion of G protein signaling membrane-bound receptors for progesterone with possibilities of transactivation to PR-B (113). Further studies are awaited to fully resolve all these matters, notwithstanding some concerns regarding anti-PR receptor antibody specificity (127, 196). There is a clear need to determine whether this type of functional progesterone withdrawal occurs at preterm labor and therefore represents a potential future pharmacological target to prevent preterm birth.

A third theoretical mechanism of progesterone withdrawal concerns metabolites of progesterone. The placenta, fetal membranes, and decidua have a prodigious capacity to metabolize progesterone into a multitude of metabolites. Rather than simply reducing progesterone concentrations, it is proposed that these metabolites may be biologically active in their own right. Several of these metabolites have more potent relaxation effects on myometrium than does progesterone (144, 163) and thus may function as important mediators of the “progesterone block” in pregnant women. Alternatively, these metabolites may compete with progesterone for binding to PR-B, although this has never been demonstrated experimentally. According to another theory, progesterone metabolites may serve as ligands for other receptors, such as the γ-amino butyric acid receptor, the OTR, or orphan nuclear receptors such as pregnane X receptor or constitutive androstane receptor (92, 144, 170). In this manner, changes in the stimulating or relaxing effects of the metabolites may accomplish a “progesterone withdrawal” without changes in maternal progesterone concentrations. To date, there is no compelling evidence to accept or discard this theory.

A final potential regulation point for progesterone bioactivity may occur at the postreceptor level and involve other transcription factors or transcriptional coregulators. A decrease in an essential factor in PR-B-mediated effects or an increase in a factor that inhibited the effectiveness of PR-B could accomplish a progesterone withdrawal. Recent studies (51) have noted changes in such coregulators as steroid receptor coactivators 2 and 3 and cAMP response element binding protein (CREB) binding protein in the uterus at parturition. Furthermore, direct interactions between PR and other transcription factors such as nuclear factor (NF)-κB also may influence uterine contractility (111), but as yet there has been no consensus as to the role these factors may play in parturition (138).

The immune system and parturition. It was noted several decades ago that labor is an inflammatory process, whether at term or preterm (125). Recent research has invested heavily in the relationship between the immune system and parturition. This has been the subject of many recent reviews (114, 154, 160, 181). Despite this, the relationship between the immune system and parturition remains quite unclear. Two essential considerations are of paramount importance when regarding this relationship. The first is the clear distinction between evidence of association and evidence of a cause-effect relationship. The second, and perhaps more important consideration is the distinction between infection and an inflammatory response. There is unequivocal evidence that significant intrauterine infection can cause preterm birth (87). However, in this clinical situation, prompt delivery is indicated and delay of parturition is not a consideration. This is in contrast to “spontaneous” preterm birth (see below), where prolongation of gestation may be beneficial and the evidence for an immune system response is less clear.

We suggest the magnitude of the inflammatory reaction may be an important determinant of its role in parturition. The pioneering studies of Romero (183, 184, 189) and others have documented concentrations of proinflammatory cytokines in the amniotic fluid of women, both at term and preterm and in the presence and absence of positive amniotic fluid bacterial cultures. Interleukin (IL)-1β concentrations in women with preterm labor and negative cultures were uncommonly elevated (only 3/42 were >250 pg/ml). However, with a positive amniotic fluid culture, the median levels were extremely elevated and 27/35 cases had values >1,000 pg/ml (183). At term, concentrations were significantly increased (3- to 4-fold) with the onset of labor (159) and were increased an additional fourfold in the presence of a positive culture (183). A similar pattern was seen for IL-6 concentrations in amniotic fluid. Concentrations increased from a median of 1.7 ng/ml (range 0.1–69.1 ng/ml) with negative cultures to 35.9 ng/ml (32.8–62.8 ng/ml) with positive cultures (189). There also was a significant increase in amniotic fluid IL-6 with the onset of normal labor at term (159) and a progressive increase through labor from median concentrations of 0.6 ng/ml at <2 cm dilation to 13.0 ng/ml at >6 cm (116).

Similar patterns were measured for amniotic fluid concentrations of tumor necrosis factor (TNF)-α. This cytokine was detected in only 4/39 women with preterm labor in the absence of infection but was detected in 12/13 women with positive cultures with a median of 820 pg/ml (183). At term, there was...
again a significant increase with the onset of labor (159). In another study, TNF-α was undetectable before labor, but following labor onset, TNF-α was present in 14/69 women in the absence of infection and increased significantly to 7/15 with positive amniotic fluid culture (184).

These data strongly support the notion that an inflammatory process accompanies labor. In normal term labor or in spontaneous preterm labor (absence of infection), this process is of moderate proportion. However, in the presence of intrauterine infection, the proinflammatory reaction is markedly augmented. Thus the animal models that utilize bacteria or bacterial products may be relevant for infection-associated preterm birth, which is the subgroup where current knowledge supports expediting delivery of the fetus at any gestation. However, it remains unclear whether these models are relevant for the spontaneous preterm birth syndrome, which is the group accounting for 40–45% of preterm births (86, 182), where improved management to prolong gestation could significantly improve infant outcomes. The article by Romero et al. (182) eloquently describes the multifactorial nature of this syndrome and the potential role played by the immune system. There is an urgent need for identification and enhanced understanding of the relationships between the immune system and mechanisms of parturition in the spontaneous preterm birth syndrome. The results will provide more effective strategies to prevent or more successfully manage this important syndrome.

Even for the spontaneous preterm birth syndrome, the most commonly considered stimulus to the immune reaction that leads to preterm labor is occult infection. However, an important negative factor regarding the cause-effect role of occult infection in this syndrome is the relatively limited effectiveness of antimicrobial agents to delay delivery. A recent meta-analysis of multiple randomized controlled trials of the use of antibiotics for spontaneous preterm labor showed neither prolongation of gestation nor significant maternal or fetal benefits in the absence of previously ruptured membranes (107, 122). An authoritative editorial also has emphasized the futility of administering antimicrobials for either treatment of preterm labor or the prevention of preterm labor in women with bacterial vaginosis (202). Moreover, follow-up studies suggested an increased incidence of childhood cerebral palsy and other neonatal complications in treated groups (118, 202). On the other hand, antimicrobial therapy may lengthen gestation and provide benefit to the newborn in the 25–30% of cases of preterm labor that follow rupture of the membranes, where there is stronger evidence for a role of microbial involvement (86, 117, 202). It is likely that clinically beneficial advancements in this area must await developments in research in other fields such as immunobiology, which are not necessarily specifically focused on the pregnant uterus.

Many proinflammatory cytokines initiate signaling pathways that include transcription factors such as NF-κB, CAAT-enhancer binding protein β-isofrom (CEBPβ), activator protein-1 (AP-1), and specificity protein-1 (Sp-1). Response elements for these transcription factors are commonly found in the regulatory regions of genes that are thought to be important in the cascade leading to parturition (OTR, FP, PGHS-1, PGHS-2, CX-43). Many more studies have explored the roles of proinflammatory cytokines on the expression of, and signaling pathways associated with, these factors. The results have been mixed, with many positive and some negative findings. One downstream effect of several proinflammatory cascades is the generation of PGF$_{2α}$. Whereas this causes luteolysis and subsequent progesterone withdrawal in rodent models, the role of PGF$_{2α}$ in the human is much less clear. Finally, one of the more consistent findings in recent microarray experiments investigating molecular events of parturition is an increase in expression of genes associated with an immune response (20, 98). In summary, these studies reinforce the association between immune activation and birth and provide some indirect and equivocal support for the cause-effect relationship.

Several studies in animal models have been designed to address a cause-effect relationship between the proinflammatory branch of the immune system and parturition. Assessment of these studies requires attention to the second consideration noted above: the distinction between infection and an inflammatory process. This distinction is not easy. Multiple commensal organisms commonly inhabit the lower genital tract in normal pregnancies. Inflammatory reactions occur in essentially all cases of labor. Other than the presence of clinical signs and symptoms of infection, the distinction between the physiological and pathophysiological environment is usually unclear. Experimental models utilizing lipopolysaccharide (LPS)/microbial extracts or pharmacological doses of proinflammatory cytokines are likely more relevant to infection-induced labor rather than the spontaneous preterm labor syndrome, yet in such instances there are distinctions even between rats and mice (see below). It is therefore critical when evaluating the literature to consider the relevance of the experimental model to the interpretation of the results and conclusions reached.

In mice, intraperitoneal injection of LPS or intrauterine instillation of killed Excherichia coli bacteria induced preterm parturition accompanied by an increase in maternal serum IL-1 concentrations (71, 103). Infusion of IL-1α to mice caused parturition that was blocked by predadministration of IL-1 receptor antagonist (185, 188). Of interest, intraperitoneal injection of LPS in mice, or transcervical inoculation with LPS to induce ascending infection in rabbits, resulted in reductions in maternal serum progesterone concentrations that preceded parturition (71). Even in primate models, information is equivocal: infusion of IL-1β into the amniotic fluid of rhesus monkeys during the preterm period caused marked increases in intra-amniotic TNF-α as well as PGE$_2$ and PGF$_{2α}$ (12). Although this was accompanied by premature uterine contractions, fetal delivery did not occur in 8 of 11 animals. Again, these models appear to be more relevant to infection-induced preterm labor than to the spontaneous preterm labor syndrome. In contrast, we infused recombinant IL-1β or TNF-α intraperitoneally into late pregnant rats to achieve concentrations in maternal blood and amniotic fluid that were similar to those measured in normal term delivery or preterm delivery in the absence of infection. There was no effect on uterine contractility or expression of genes characteristically associated with parturition in this species (146).

**Target systems.** Two distinct and equally important physiological processes contribute to the final stages of parturition and fetal delivery: generation of uterine contractions and ripening of the cervix. For the former, we address regulation of the relaxatory/contractile mechanisms of uterine myocytes and the signal transduction pathways thought to be important in regulating uterine contractility. For the latter, we focus on the cellular mechanisms...
that transform the firm rigid cylindrical cervix of early pregnancy into the soft, distensible tissue that can be stretched or dilated to allow passage of the products of conception through the birth canal.

**Contractile apparatus of the uterine myocyte.** Myometrial contractile effort in all species is periodic and arises from spontaneous action potentials due to unstable membrane potential. These have been recorded in vitro in humans, rats, rabbits, mice, and guinea pigs, and although there are some configurational differences across species, these likely reflect divergent ion channel expressions or biophysical profiles. It is accepted that their purpose is to elevate the intracellular Ca\(^{2+}\) that is a prerequisite for contractile activation. The Ca\(^{2+}\) increase arises from transsarcolemmal extracellular Ca\(^{2+}\) entry and/or release of Ca\(^{2+}\) from the intracellular store in the sarcoplasmic reticulum. As term parturition approaches, there is an increase in the gap junctional protein connexin43 (Cx43), gap junction channels as viewed by electron microscopy, and electrical conduction parameters, all of which would enhance the ability of any elevations in Ca\(^{2+}\) to be spread among neighboring cells.

These action potential-driven contractions can be augmented by uterotonic G protein-coupled receptor (GPCR) stimuli (Fig. 3) such as OT, ET-1, and PGFs\(_{2\alpha}\) (226). These stimulating GPCRs are characterized by having a Go\(_q\) subunit in the heterotrimeric G protein complex. Augmentation of contraction arises from two physiological mechanisms. The first is through accessing additional pathways of Ca\(^{2+}\) availability for contraction over and above that occurring with spontaneous action potential generation alone. These include 1) receptor-operated sarcolemmal Ca\(^{2+}\) entry, 2) Ca\(^{2+}\) release from the sarcoplasmic reticulum consequent to G protein-mediated phospholipase C (PLC) hydrolysis of membrane phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) into inositol 1,4,5-trisphosphate (IP\(_3\)) generation, and binding to IP\(_3\) receptors (IP\(_3\)R) to release stored Ca\(^{2+}\) into the cytoplasm, and 3) store depletion-mediated Ca\(^{2+}\) entry. The increases in Ca\(^{2+}\), whether occurring spontaneously or agonist mediated, bind to calmodulin, which in turn activates myosin light chain kinase (MLCK) that phosphorylates the regulatory 20-kDa myosin light chains (MLC\(_{20}\)) to initiate the contractile mechanism of the myocyte. Action potential cessation and reduction in Ca\(^{2+}\) enables relaxation through dephosphorylation of MLC\(_{20}\) by a myosin light chain phosphatase (MLCP).

The second mechanism of augmentation of contractile activity occurs by increasing the sensitivity of the contractile apparatus to Ca\(^{2+}\). Simultaneous measurements of Ca\(^{2+}\) and contractility in rodent or human uterine tissue strips in vitro illustrate that uterotonins can induce this phenomenon (210, 225). In general, Ca\(^{2+}\) sensitization mechanisms result in inhibition of MLCP, which then favors prolongation of the phospho-MLC\(_{20}\) activity with enhancement of myocyte contractility. Although considerable progress has been made in elucidating the molecular details of such pathways in vascular smooth muscle (206), they remain unresolved and somewhat contentious in uterine smooth muscle. In addition to IP\(_3\), an additional second messenger molecule is generated from PLC-

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**Fig. 3.** Some basic pathways of signal transduction in uterine myocytes. Stimulatory signals, such as those initiated by oxytocin (OT), endothelin-1 (ET-1), or prostaglandin F\(_{2\alpha}\) (PGF\(_{2\alpha}\)), are usually mediated through G protein-coupled receptors (GPCR) that are linked through Go\(_q\) subunit to phospholipase C (PLC). PLC catalyzes conversion of membrane phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) into inositol 1,4,5-trisphosphate (IP\(_3\)) and diacyl glycerol (DAG). IP\(_3\) stimulates Ca\(^{2+}\) release from the sarcoplasmic reticulum (SR), and this in turn causes increasing Ca\(^{2+}\) influx from the extracellular space. Ca\(^{2+}\) flux through the plasma membrane occurs through specialized channels that may be voltage-, receptor-, or store-gated. The increased Ca\(^{2+}\) activates calmodulin (CaM), which then stimulates myosin light chain kinase (MLCK) to phosphorylate myosin light chains (MLC) to initiate activity of the contractile machinery. The contraction is terminated by the activity of MLC phosphatase (MLCP) by removal of the phosphate from phospho-MLC. Stimulatory GPCRs also may activate the small monomeric GTPase RhoA, which, after binding GTP, will activate Rho-associated kinase (ROK). The action of ROK is to inhibit MLCP and thus enhance concentrations of phospho-MLC and potentiate the contractile activity produced by the increased Ca\(^{2+}\). This is termed Ca\(^{2+}\) sensitization. The DAG may activate protein kinase C (PKC), which may then phosphorylate and activate a 17-kDa protein (CPI-17) that can directly inhibit MLCP and contribute to Ca\(^{2+}\) sensitization. Inhibitory signals to uterine myocytes also are commonly mediated through GPCRs, but these are linked through Go\(_s\), which stimulates generation of cAMP to activate protein kinase A (PKA). PKA may have a variety of inhibitory effects, but the best characterized is the phosphorylation of MLCK to prevent interaction with and activation by Ca\(^{2+}\)-CaM complexes.
mediated hydrolysis of PI(4,5)P2. This is diacyl glycerol (DAG), which activates several isoforms of the protein kinase C (PKC) family. The effects of PKC stimulation in uterine myocytes are myriad, with uncertain biological implications (8). However, uterine tissue contains the small protein CPI-17 (PKC-potentiated inhibitor of protein phosphatase 1c), which, when activated by PKC, is a potent inhibitor of MLCP (162). Much recent attention has been given to rhoA-Rho-associated kinase (ROK)-dependent pathways mediating agonist-induced Ca2+-sensitization in vascular smooth muscle, but the exact involvement of ROK pathways in Ca2+-sensitization of uterine contractions remains debatable (120, 123, 225). The contribution in the uterus of other molecular signaling pathways suggested generally to participate in smooth muscle Ca2+-sensitization is really unresolved.

The best-characterized relaxant system in the uterine myocyte is that involving adenylyl cyclase (AC)-cAMP-PKA (Fig. 3). Again, the agonists for this pathway, including most notably the β2-adrenergic agonists and PGI2, stimulate GPCRs (5, 7). However, in this case, the receptors are linked through Gs to stimulate AC, which results in formation of cAMP that will activate protein kinase A (PKA). As with PKC, the intracellular substrates and physiological effects of PKA are many and varied (169). In the uterine myocyte, it appears the major action is to phosphorylate MLCK at the calmodulin-binding site and thus inhibit phosphorylation of MLC (208), but other mechanisms also are possible. Although β2-adrenergic agonists have failed to offer suitable benefits as tocolytics, there are continuing efforts at targeting cAMP generation for this purpose, most notably in the application of β3-adrenergic agonists or phosphodiesterase inhibitors (63). Another potential uterine relaxant system is NO produced by inducible nitric oxide synthase (iNOS) (13, 178). NO activates guanylyl cyclase to produce cGMP, and exogenous agents that raise intracellular cGMP levels induce uterine relaxation in vitro (61). Divergent and convergent pathways of cyclic nucleotide action may exist. However, the necessity of NO-mediated uterine relaxation to be coupled to cGMP generation has been questioned (28).

Whether linked through Goq or Goq, the contractile effects resulting from GPCR stimulation occur in a time course of seconds to minutes. However, in recent years there has been increasing interest in the possibility that GPCR-stimulated signaling, whether resulting in increased Ca2+ or activation of PKC or PKA, may mediate longer term effects through activation of transcription factors regulating transcription of specific genes (70). These effects may occur over a time course measured in hours or days.

CERVICAL RIPENING. The morphological transformation of the cervix is one of the most remarkable events in the process of parturition. It is not clear how much overlap or coordination exists in the mechanisms regulating the changes in the contractile properties of the fundus and the mechanical properties of the cervix. During most of pregnancy, the cervix is a rigid cylindrical organ usually of 4–7 cm in length. In the first pregnancy, the canal is essentially closed. In pregnant women who have previously had a vaginal delivery, the canal may be somewhat shorter and as much as a centimeter in diameter. The rigidity of the cervix is due largely to its high content of collagen, particularly types I and III. Over the course of late pregnancy, this rigid cylindrical organ becomes softer and increasingly distensible, a process termed cervical ripening. During the process of parturition, the cervix will completely efface and dilate to allow passage of the fetus and placenta.

The pioneering studies of cervical ripening focused on changes in connective tissues (59, 124). Most of the research concerning the regulation of cervical ripening has focused on the same pathways that have predominated contractility research: the role of progesterone and the influence of the immune system. Again, the most commonly used animal models have been rats and mice. Around the time of parturition, there is increased expression and activity of matrix metalloproteinases (MMP), leading to progressive breakdown of the collagen matrix with a concomitant increase in water content, hyaluronan, and glycosaminoglycan concentrations in the cervix (90, 119, 124, 175, 217). More recently, the use of light-induced autofluorescence (LIF) measurements with a Collascope has, particularly in rats, given information on the in situ changes in cross-linked collagen indicative of cervical ripening with parturition (136).

In mice, the process of cervical softening begins in mid-pregnancy, approximately gestational day 10–12 (25, 174). However, there is an accelerated phase, labeled cervical ripening, that occurs in the last two days of gestation. The latter event is accompanied by an influx of immune cells, particularly neutrophils, into the cervical matrix (228). Intrauterine application of LPS will cause a similar influx of immune cells into the cervix, and this can be prevented by prior treatment with a synthetic progestagen, but not with progesterone itself (229). Although it is not surprising that a potent inflammatory stimulus such as LPS causes an influx of inflammatory cells into an adjacent tissue, the results do support a cause-effect relationship between these cells and cervical ripening. The process of parturition in pregnant women also is accompanied by an influx of proinflammatory cells into the cervix (153).

Partition Studies in the Human

Obstacles to the study of human parturition. An ideal circumstance in our goal to investigate the mechanisms of human term and preterm labor would be having the ability to accrue all essential experimental details from human-derived tissue. However laudable, this goal is unrealistic. In animal models it usually is possible to get optimal experimental samples at precisely calculated time points. In contrast, collection of the most appropriate human samples is rigidly regulated by clinical, ethical, and even economic considerations. From a practical viewpoint, some fetal tissues (umbilical cord, amnion and chorion membranes, and placenta) are relatively easy to obtain following delivery. On the other hand, maternal tissues are much more difficult. Adherent maternal decidual tissue can be harvested from the chorionic membrane following vaginal delivery. However, this tissue represents only the superficial layers of decidua and may not totally represent the basal decidua, which lies adjacent to the myometrium and thus may have a more important regulatory influence over this target tissue. Furthermore, these samples are often contaminated with chorionic membrane tissue (168). Although distinction between chorion and decidua can be made by use of pure chorion laeve obtained from the inter-sac membrane of dichorionic, diamnionic twin gestations (18, 85), this represents only a very specific situation with limited relevance. At the time of cesar-
Clinical practice. More recently, an American Nalodological deficiencies, and the findings were never widely treated group (109). This trial was criticized for several meth-
essentially eliminated the occurrence of preterm labor in the 17-hydroxyprogesterone acetate as the therapeutic agent and preterm delivery. The earliest randomized control trial used progesterone supplementation in the prevention of human pregnancy remains essentially eliminated. The role of progesterone in human pregnancy remains unclear. Animal studies have provided strong evidence that PGF2α produced by the endometrium has a critical role in causing luteolysis and subsequent progesterone withdrawal, thus initiating parturition (95). However, the data are not so strong in animals or humans that PGs have an important direct contractile effect on the myometrium (9, 179, 226). It also appears clear that stimulation of PGHS is a major component of the immune response that accompanies labor, and it is possible that PG products are important in coordinating the influences of the immune system on the process of parturition. One of the potential mechanisms, for which there is some experimental support, is in stimulation of myometrial gap junction formation that enables rapid and efficient spread of electrical impulses through the uterine muscle to facilitate the strong, coordinated contractions characteristic of active labor (79). In animal models where gestation is dependent on the corpus luteum, an immune response, whether initiated by infection or other stimuli, could lead to generation of PGF2α, subsequent luteolysis, and parturition. However, in animals where progesterone is synthesized predominantly in the placenta, there currently is no evidence that this mechanism is operative or physiologically relevant. This suggests that the requisites for an animal model for human parturition should include the placenta being the predominant source of progesterone in gestation.

Whereas antiprogestins terminate pregnancy at any stage of pregnancy in the well-studied, progesterone-dependent animal...
models, there is relatively little information regarding the effects of antiprogestins in mid and late human pregnancy. Some data suggest that antiprogestins, although unable to produce abortion by themselves, cause ripening of the cervix and increase sensitivity to contractile prostaglandins during induced abortions in the second trimester of human pregnancy (214). A Cochrane review of clinical trials in 2000 suggested that the antiprogestrone RU-486 increased the incidence of cervical ripening and spontaneous delivery within 48 h in women at term (150). It also has been noted that a significantly lower amount of OT is required to induce labor in women at term who have been treated with RU-486 compared with placebo (75). Thus a functional progesterone withdrawal, or at least a pharmacologically induced functional progesterone withdrawal, may play some role in cervical ripening and in increasing myometrial sensitivity to contractile stimulants. In this regard it is interesting to note the suggestion that any benefits of progesterone-based therapy for delaying preterm labor may reside not at the level of tocolysis, per se, but at the level of inhibiting cervical shortening and ripening, possibly via anti-inflammatory actions (see below) (228).

Many studies have assessed the appearance of contraction-associated proteins in the uterus of pregnant women in late gestation with variable and conflicting results. We have demonstrated estrogen-stimulated synthesis of OT mRNA and peptide in maternal decidua with evidence that production rates increase at the onset of parturition (41, 42). Some investigators found that concentrations of OTR were increased in both decidua and myometrium before labor onset (77), whereas others found that they were not changed (98) or were reduced (164). Using in vitro techniques, several investigators have demonstrated that proinflammatory cytokines cause an increase (213) or decrease (100, 173, 199) in the expression of the OTR gene in uterine myocytes. The FP receptor appears to have decreased expression during pregnancy and then increases near labor in some studies (21), but not all (94). The increase may be region specific (21). It is quite likely that the variability and contradictory nature of these data are due in large part to the obstacles in collecting human tissues samples and the wide variety of in vitro approaches noted above.

Several recent studies have utilized microarray techniques to assess changes in the human uterus that occur at the time of parturition. The huge amount of data generated by this technology and the associated significant bioinformatical complexities have provided only limited understanding of the physiology of parturition. Few samples have been studied, and even then, in a variety of different experimental paradigms (20). Comparisons have used between myometrial tissues from nonpregnant women and women in mid and late pregnancy, before or after labor onset. Depending on the cutoff definitions used, significant changes in expression occurred in genes associated with the immune system and in a variety of signaling pathways, particularly the mitogen-activated protein kinase pathway (20, 98, 135). Surprisingly, those involved with Ca<sup>2+</sup> signaling pathways and the uterine myocyte contractile apparatus were generally not increased, albeit the absence of transcript changes on arrays cannot be taken as evidence of a lack of importance and may simply reflect low sensitivity of detection. Nonetheless, continued improvement in genomewide screening technologies means that it will be important in the near future to establish standard approaches for the appropriate use and interpretation of this powerful technology in the field of parturition: in particular, a standardization for comparison of large-scale data sets across species. In the field of discovery research, it will be necessary to provide clear rationale for the specific design of the studies and a definitive plan for management and application of the data obtained.

Cervical ripening is a clinically important entity in human parturition. Ultrasound assessment of progressive cervical shortening in pregnant women preterm is a strong predictive marker of preterm labor onset in either asymptomatic patients or those with a previous history of preterm labor (52). Failure of the cervix to properly ripen is a common cause of failed vaginal delivery, particularly in situations where induction of labor before term is indicated by the medical condition of mother or fetus. The most commonly used agent to ripen the cervix is local application of prostaglandin E or an analog (30, 62). The mechanism of action is suspected to be stimulation of activity of MMPs or increased permeability of the cervical vasculature, facilitating influx of inflammatory cells (88, 172). As with the fundus, there is an influx into the human cervix of cells of the immune system, particularly neutrophils, at the time of cervical ripening (115, 161). These cells are likely sources for proinflammatory ILs and MMPs that participate in remodeling of the cervix (110, 124). We noted previously the cervical ripening effects of treatment with antiprogestins late in human pregnancy. Cervical application of NO donors also can stimulate an influx of immune cells and cervical ripening (47).

**Limitations and Benefits of Currently Favored Animal Models**

In this section, we present a brief overview of the essential features of animal models that are in current use. For each of the models, there are excellent review articles available. The overview follows a chronological sequence and includes a critique of the advantages and disadvantage of each of the models. Table 1 provides a summary of important reproductive features of each species relating to the suitability of the model for translation to the human.

**Sheep.** The seminal studies of Liggins (125) and others unraveled the fascinating details of the regulation of parturition in this species. Indeed, these studies established the basic understanding of parturition on which subsequent experimentation was based. Progesterone is essential for ovine pregnancy maintenance. The major source of progesterone is the placenta, and the mechanism controlling progesterone withdrawal involves a rather complex but fascinating mechanism. In the sheep, the fetus plays a critical role in the endocrine mechanisms that regulate the timing of parturition. With maturation of the fetal hypothalamic-pituitary axis, there is increasing secretion of adrenocorticotropic hormone (ACTH) into the fetal circulation. This stimulates the fetal adrenal gland to increase production of cortisol, which has two remarkable functions with respect to the birth process. First, it stimulates functional maturation of the fetal lungs and other systems. Second, it stimulates increased expression of the steroidogenic enzyme P450<sub>C17</sub> in the placenta. This enzyme enables the placenta to convert progesterone through to estrogen. Hence, as the substrate progesterone is metabolized, maternal progesterone levels fall dramatically while estrogen concentrations increase. A particularly striking and possibly unique feature of...
this model is its natural efficiency: the same system that induces maturation of fetal lungs and other organs also initiates the process of parturition. Models of preterm birth in the sheep can be established by administration of progesterone antagonists or by infusion into the fetus of corticotrophin releasing hormone (CRH), ACTH, or glucocorticoid. These models have contributed significantly to our understanding of parturition and fetal development. Although this model is clearly not applicable to the human, the unique dual features of the same pathway leading to fetal maturation and parturition gives rise to an interesting possibility. An analogous concept in the human could involve activation of a single pathway that could activate both the mechanisms of parturition as well as the proinflammatory response required for the healing/involution process following delivery.

The major advantages of the sheep model relate to the size of the animal, which allows instrumentation of the mother and fetus during the latter part of gestation. This facilitates the longitudinal sampling that is essential to precisely determine changes in physiological systems and explore their regulation around the time of parturition. Conversely, the ovine model appears to have two major discrepancies from the human, which limit its usefulness for studies of parturition. The first is its dependence on withdrawal of progesterone. The second is the pivotal role of the fetus, which appears not to be the case in human pregnancy. In the human, fetal death does not appear to have a direct effect on the length of gestation. Of more concern is the lack of effectiveness of glucocorticoids to initiate parturition in human pregnancy. Because of their effect on accelerating fetal lung maturation, large doses of glucocorticoids are routinely administered to pregnant women who are in threatened preterm labor. This treatment has been studied in many randomized placebo-controlled trials and has been demonstrated to have beneficial effects on several fetal systems, including the lungs and perhaps the central nervous system and the gut (23). However, there is no shortening of the interval to delivery in the glucocorticoid-treated women compared with those who received placebo treatment. This strongly supports the notion that glucocorticoids do not have a major role in the process of parturition in the human. The ovine model will continue to be very important in understanding regulation of fetal growth and physiological development during pregnancy but is likely to be of limited use to study parturition per se relevant to the human.

**Rabbits, rats, and mice.** These models differ from the sheep in two fundamental ways. In all of these animal models, progesterone is produced by the corpora lutea of pregnancy and not in the placenta. However, progesterone withdrawal remains necessary and sufficient to evoke parturition. The second major difference from the sheep is that the fetus appears not to play such a dominant role. The progesterone withdrawal is a result of luteolysis, which results from the actions of PGF₂α produced in the endometrium. In all these models, preterm birth can be precipitated with antiprogestins, and this can be inhibited with supplemental progesterone (48, 66, 171). The original “progesterone block” theory was supported mainly by data from rabbits (53). More recently, rats and mice have become the more commonly used models.

Several strains of rats have been utilized in studies of parturition. There appear to be some similarities between the rat and humans regarding some of the molecular pathways regulating uterine contractility in terms of molecules that are upregulated (OTR and Cx43, among others) and downregulated (participants of the cAMP-PKA signaling axis and iNOS, among others) (9, 66). The major advantages of this model include the relatively short gestation period, low cost, and a reliable method for preterm labor induction. In addition to progesterone, estrogen appears to be important in the timing of parturition, since treatment with an estrogen receptor antagonist delays parturition by 1 day and may cause relative fetal growth restriction (68). Interestingly, we (65) and others (102) have been unsuccessful in developing a reliable model of preterm delivery using LPS or *E. coli* bacteria in rats. This may have relevance to our previously noted failure to effect uterine contractility or contraction-associated gene expression by intraperitoneal injection of IL-1β or TNF-α in late gestation. Given that LPS can induce preterm labor in mice, these findings caution us against extrapolating unquestioningly between species in relation to parturient effects of infection or inflammation.

More recently, increasing attention has been devoted to mice as models for parturition because of the ability to genetically manipulate single or multiple genes. Several “knockout” studies using targeted gene disruptions of endocrine/paracrine pathways provided important information regarding the regulation of parturition. Surprisingly, complete disruption of the gene for either OT or OTR had no effect on the process of parturition, although lactation was impaired due to the absence of milk

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**Table 1. Reproductive characteristics of animal models compared with the human**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Rabbit</th>
<th>Sheep</th>
<th>Rat</th>
<th>Mouse</th>
<th>Guinea pig</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestation, days</td>
<td>32±3</td>
<td>147±4</td>
<td>22±1</td>
<td>20±1</td>
<td>67±3</td>
<td>266±14</td>
</tr>
<tr>
<td>Usual litter size, no.</td>
<td>8±4</td>
<td>1–2</td>
<td>10±5</td>
<td>5±2</td>
<td>1–2</td>
<td>3±2</td>
</tr>
<tr>
<td>Placental morphology</td>
<td>Hemochorial, labyrinthine</td>
<td>Epithelial-chorial, cotyledonary</td>
<td>Hemotrichorial, labyrinthine</td>
<td>Hemotrichorial, labyrinthine</td>
<td>Hemomonochorial, villous, discoid</td>
<td></td>
</tr>
<tr>
<td>Source of progesterone</td>
<td>Corpus luteum</td>
<td>Corpus luteum, then placenta</td>
<td>Corpus luteum</td>
<td>Corpus luteum</td>
<td>Corpus luteum, then placenta, then placenta</td>
<td></td>
</tr>
<tr>
<td>Progesterone withdrawal?</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Induction of preterm birth</td>
<td>Antiprogestin, ovarioctomy</td>
<td>Fetal ACTH, glucocorticoid, antiprogestin</td>
<td>Antiprogestin, ovarioctomy</td>
<td>Antiprogestin, ovarioctomy, IL-1β</td>
<td>Cervical ripening (PGEx or antiprogestin) plus oxytocin</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD.
letdown (151, 212, 230). Disruption of the FP receptor prolonged gestation indefinitely due to absence of the luteolytic effect of PGF$_{2\alpha}$ (209). After ovariectomy, these mice delivered normally, suggesting that, in this species, PGF$_{2\alpha}$ was essential for luteolysis but not for the subsequent process of parturition itself. Ablation of PGHS-1 also causes prolonged gestation, suggesting this isoform was responsible for the endometrial synthesis of the luteolytic PGF$_{2\alpha}$ (95, 232). Remarkably, when both the PGHS-1 and OT genes were disrupted, parturition began on time (95). This was subsequently shown to be due to the fact that OT has a significant luteotropic activity that maintains gestation in the PGHS-1-deficient mouse (108). However, when the OT gene is disrupted along with the PGHS-1 gene, the PGF$_{2\alpha}$ produced by PGHS-2 is sufficient, in the absence of OT, to cause luteolysis and subsequent parturition. The course of labor in these double-knockout animals was protracted over a 2-day period, perhaps because of absence of stimulation of the OTR. Interestingly, gestation in CRH knockout mice is normal, but the pups do not survive due to pulmonary immaturity resulting from lack of maternal or fetal glucocorticoid synthesis (147). More recently, knockout of the cannabinoid receptor 1 results in alterations in CRH, as well as estrogen-to-progesterone ratios, and induced preterm labor, suggesting that progesterone withdrawal itself may be the result of a complex series of events (220).

Surprisingly little attention has been given to phenotypic effects regarding parturition resulting from alteration of genes that might be anticipated to alter myometrial contractility. More consideration should be given to this in large-scale mutagenesis screenings. Studies of two genes are of note. First, murine overexpression of small-conductance Ca$^{2+}$-activated K$^+$ channel isoform 3 results in altered in vitro uterine contractile parameters, prolongation of normal pregnancy, and inhibited delivery in two models of preterm labor (22, 69, 165). However, the suggestion that parturition requires downregulation of these channels has yet to garner supporting evidence in the human. Second, conditional ablation of the principle myometrial gap junctional connexin, Cx43, resulted in pregnancy prolongation (64). Because Cx43 is upregulated in all species before labor, this finding supports the long-held proposition that this protein is pivotal for coordination of myometrial cell-to-cell coupling.

Gene knockout studies also have been applied to investigations of the molecular mechanistic pathways of the induced inflammatory responses preceding preterm labor. Intraperitoneal infection with E. coli causes preterm delivery in mice, and this is completely dependant on Toll-like receptor signaling adaptor protein MyD88 (72). Interestingly, mice lacking functional IL-1B receptors responded the same as wild-type mice to immune system insult (103). Thus it remains unclear whether the immune stimulus works in an entirely paracrine fashion or whether it operates indirectly, provoking a maternal systemic response. The incidence of LPS-induced preterm labor in mice is reduced by either progesterone or synthetic gestational agents (229). Although the mechanisms of action are unknown, they are accompanied by gene expression changes at the cervix, again pointing to the possible anti-inflammatory role in prevention of cervical ripening (228). Intriguingly, LPS-induced preterm labor also is abrogated by inhibition of phosphodiesterase type 4 or Rho-associated kinase (200, 211).

These animal models also have been used in the study of cervical ripening. For example, in the rabbit, vaginally administered indomethacin prolonged RU-induced preterm labor and reduced cervicovaginal MMP activity (74). In very small studies, LPS-induced delivery and cervical distension has been reduced by the potential antibacterial/anti-inflammatory actions of cervically applied recombinant human lactoferrin (97, 149). The rat also has been used in limited studies to explore regulation of cervical changes before parturition (44, 134, 223). In mice, studies of gene disruption revealed a very interesting facet of cervical preparation for mouse parturition. Knockout of the gene for the steroidogenic enzyme 5α-reductase isoform 1 caused a phenotype with delayed and impaired parturition secondary to failure of cervical dilation (128, 129). This has suggested a role for this enzyme in the process of cervical ripening, but it is not clear whether this reflects an important metabolic pathway to decrease local progesterone concentrations or whether there is a direct role for 5α-reduced steroids in the process of cervical ripening. A natural occurrence of this phenomenon has not been observed in human pregnancy.

In summary, genetic manipulation of the mouse model has confirmed the importance of several suspected pathways in parturition and has cast doubt on the importance of others. However, as with all these animal models, the dependence of parturition on progesterone withdrawal diminishes their relevance to human parturition. The gene disruptions focused on regulation of luteolysis will likely have little relevance to human parturition. Similarly, genetic manipulations that alter the responses to LPS or E. coli may or may not provide useful information regarding the human spontaneous preterm labor syndrome. Finally, considering the evolutionary importance of parturition, it is likely that multiple modular pathways (see below) have developed to regulate the process, and single gene disruptions may thereby provide information lacking a systems context. The continued approach of single/multiple gene disruption will undoubtedly, in certain situations, provide important information regarding potential roles of specific genes, but it is likely to provide only limited data relevant to the overall physiological pathways that regulate parturition in the primate.

Nonhuman primates. Higher order primates remain the most closely aligned to humans in terms of pregnancy in a number of ways: a closer gestational length than rodents, lack of maternal serum progesterone withdrawal, unicornate uterus, and electromyographic activity (80). Data garnered from these studies have been tremendously informative of parturient events, their influence in part enhanced by the deficiencies inherent in other animal models. As such, they continue to be used for in vivo studies, particularly the assessment of uterine electrical activity (194), the tocolytic action of new drugs (176), and the parturient responses to administration of inflammatory cytokines or infectious agents (12, 91, 193). For example, recent studies have explored the actions of OTR antagonists, some of which are registered for clinical use. Intravenous administration of barusiban, a selective OTR antagonist, to cynomolgus monkeys had greater inhibitory action on OT-induced early delivery than the mixed OT/vasopressin antagonist atosiban (176, 177). Intravenous infusion of bacterial product, IL-1β, or TNF-α induces preterm labor in rhesus monkeys. The IL-1β-induced preterm labor is inhibited by...
pretreatment with indomethacin to inhibit prostaglandin production, dexamethasone, or IL-10 (195). LPS-induced preterm labor was reduced or delayed by pretreatment with a Toll-like receptor 4 antagonist (1).

The marmoset is a higher order nonhuman primate that has multiple pregnancies per year. It has been utilized as a primate animal model for early pregnancy events of uterine implantation and placental organogenesis, although it appears not to have been used for parturition-related studies. Before suitability for this purpose is recommended, there should be further clarification of the limited but rather surprising suggestion, for a primate, of parturition in the marmoset being preceded by an elevation in maternal estrogen:progesterone levels (37).

Perhaps most noteworthy among the studies using primates in toxicology studies is the demonstration that 17α-hydroxyprogesterone caproate caused fetal toxicity in rhesus (but not cynomolgus) monkeys (43, 101). Although the suitability of primates as a mimic of the human situation is high, their use is accompanied by difficulties of an ethical and practical nature. The cost of such experimentation (infrastructure for housing, professional care of individual animals) is great and is considered prohibitive to many grant-awarding bodies. Such work is presently only accessible to a few researchers in select institutions. For the vast majority of experimental work, researchers therefore require access to more pragmatic alternative tissue sources, and this has driven the use of nonprimate models of parturition and preterm labor.

Conceptual Comparisons Between Human and Animal Models

On the basis of the foregoing literature, we believe there are two major conceptual deficiencies in currently used animal models. The first relates to the essential progesterone withdrawal in the animal models, not in the human. The second concerns the sequential timing of the processes involved with uterine activation.

Progesterone withdrawal. In all the animal models described in the previous sections (excluding primates), parturition is the end result of an apparently linear sequence of physiological events. Depending on the model, this includes initiation of luteolysis or a change in pattern of placental steroidogenesis followed by a significant withdrawal of progesterone from the maternal circulation (Fig. 1). By contrast, the best evidence suggests maternal serum progesterone does not play such a dominant role in human parturition. Although there may be some degree of “functional progesterone withdrawal,” as discussed above, this appears to be neither sufficient nor perhaps necessary for human parturition. Thus the entire physiological basis for uterine activation and parturition in the vast majority of currently used models may be overly simplified. Clearly, the most applicable animal model for human parturition must address the complexities of the mechanisms that regulate the timing of uterine activation and parturition, including, but not limited to, those associated with progesterone withdrawal.

Modular accumulation of physiological systems. The second, major deficiency may be linked to the first and involves the chronology of the events of uterine activation and parturition. In the commonly used models, the critical transition from quiescence to activation is relatively abrupt, giving rise to the concept of a trigger for parturition. Given normal biological variability, the short time frame of the process of uterine activation in rats and mice makes it difficult to distinguish any temporal sequence of recruitment of physiological systems that constitute the process of parturition. However, this apparently simple sequential mechanism operates very efficiently, and “spontaneous” preterm birth is a rare event in these species. Conversely, in the human there is a longer gestation period and less convincing evidence of any “trigger” mechanism such as a major steroidal switch. The critical transition from uterine quiescence to activation may be a more gradual, complex, and multifactorial process. This increased complexity may underlie the relatively common occurrence of spontaneous preterm birth. We suggest that, in the human, the trigger concept may at worst be misleading and at best an oversimplification.

We are proposing a new alternative concept, which we have attempted to illustrate in Fig. 4. For the purposes of this review,
the focus is the occurrence of spontaneous preterm birth that occurs over a broad interval (20–36 wk of gestation) in the absence of pathology such as infection or uterine abnormalities, including cervical incompetence. We reason that for preterm birth to occur, the physiological systems, which we refer to as modules, necessary for birth must become operative over that broad time span. Each module may be controlled by its own regulatory mechanisms (genomic and nongenomic). The wide variety of determinants of preterm birth suggest that there may be an equally diverse range of such modules, some known and others likely yet unknown. Such widespread activity is not unexpected, given the tremendous amount of cellular remodeling occurring over the last half of pregnancy to accommodate the growing fetus and to prepare for the eventual transition from the quiescent to contractile phenotype. We propose that before the time of birth, there is an integrative and synergistic coordination of a critical mass of these modules to achieve the contractile phenotype. In normal circumstances, this occurs at term. However, in abnormal circumstances, this may occur anytime preterm. We have termed this concept modular accumulation of physiological systems (MAPS) (9).

In the case of preterm birth, the modules required to achieve the contractile phenotype may not be identical to those usually occurring at term, as long as the critical mass is obtained. Some particular modules may contribute considerably more than others toward achievement of this critical mass. The modules may be situated in different tissues and likely encompass interactions between decidua and myometrium as well as other tissues. Myometrial modules will include, among many others, concentrations of the protein components of contractile filaments; interactions between signaling intermediaries impinging, positively and negatively, on components of the contractile apparatus; and ion channels and other mechanisms involved with regulation of intracellular Ca\(^{2+}\) concentrations or Ca\(^{2+}\) sensitization. Other uterine modules will include endocrine/paracrine production of uterine stimulants and relaxants, as well as their receptors, and signaling pathways that regulate the activities of transcription factors, translation, or posttranslational modifications. It is necessary to understand the module complexities at several levels. This includes knowledge regarding physiological processes in single cells (e.g., the regulation of Ca\(^{2+}\) homeostasis) as well as intercellular interactions of a homocellular (e.g., action potential propagation between myometrial cells) or heterocellular nature (e.g., paracrine interactions between decidual and myometrial cells). In addition, it also will be necessary to consider tissue regionalization (fundal myometrium vs. lower uterus) as well as the physiological function of the whole organ (e.g., coordination of events from the decidual-fundal interface to lower myometrial-cervical partition).

We believe that the MAPS concept provides a more useful schematic for the events that lead to labor in the human than the paradigms of a singularly “triggered” mechanism, which were derived from studies in animals with rapid withdrawal of progesterone. The transition from quiescence to activation is viewed more as an evolution as opposed to a switch. Rather than seeking the trigger, experiments need to be designed to investigate synergistic interactions among a variety of physiological systems and tissues.

A hallmark of the MAPS concept is that multiple systems, both contractile and relaxant, will be integrated synergistically by a variety of stimuli, compatible with the diverse etiological factors that precede preterm labor. Integrated organ systems are often suggested to contain significant elements of molecular redundancy. Multiple gene transcripts, proteins, or pathways may develop over a common time course but without a clear indication of a pivotal purpose. It is now emerging that spatiotemporal signal integration may be rather more purposeful and less wasteful than formerly considered (152). In fact, the situation of the gravid human uterus might actually be evidence of a biological systems robustness designed to efficiently orchestrate parturition and uterine involution. As noted above, this may help explain why we have yet to obtain a broadly effective tocolytic drug for preterm labor treatment: if one component of a multimodal system is inhibited, then eventually a parallel module(s) overcomes this deficiency to achieve the desired biological aim (which of course, may not be the desirable medical outcome in the case of spontaneous preterm labor).

Indeed, a particularly compelling feature of our MAPS paradigm for human labor may be an interweaving of the procontractile and proinflammatory systems. We speculate that evolutionary forces have adapted the maternal organism to prepare not only for the huge myometrial contractile effort to deliver the fetus and placenta but also for the attendant tissue destruction, decidual shedding, and extravasation of blood. Thus part of the MAPS process, in addition to integrating uterine contractile activity, may be to initiate preparedness of the immune system for healing/remodeling within the uterus immediately following birth. Although many experiments have demonstrated the temporal association between transformation of the uterus to an activated state and increased activity of the immune system, the physiological relationships between these events remain unclear. We have attempted to illustrate this in Fig. 5. Thus we should be mindful that many signaling modules will be operating in parallel and that spatiotemporal regulation of these systems will be complex. In the absence of clear evidence of a fundamental, singular trigger of human parturition, it remains possible that the interrelationships between the modular minisystems of the proinflammatory and procontractile parturient processes may be of a consensual nature. An accumulation, merging, or crossover of permissive but previously distinct signaling pathways results in the amplification of the biological response (in this case, uterine activation) and less cause-and-effect than is often presumed.

The potential complexity of a MAPS model requires considerable validation that is unlikely to be accomplished satisfactorily from only human biopsy material. Again, this will necessitate validatory experiments in a suitable animal model.

Proposal of the Guinea Pig as a Model for Human Parturition

In the preceding sections, we have attempted to identify the major concerns with currently used models of parturition. On this basis, we propose that the requirements for a better animal model include the following. 1) Progesterone should be produced predominantly in the placenta, and withdrawal of progesterone from the maternal circulation should not be the critical stimulus to parturition. 2) The transition from uterine quiescence to activation should occur over a time span sufficiently long to facilitate longitudinal assessment of the identity...
and ontogeny of the physiological systems involved. The natural occurrence of spontaneous preterm delivery would support the similarity of the model to the human. 3) The size of the animal should enable longitudinal sampling of endocrine parameters in the same animals throughout gestation and parturition. 4) The cost of the model, including purchase price, ease of time-mated breeding, maintenance costs, and experimentation costs, among others, must enable collection of tissues at multiple time points in gestation to investigate, physiologically and biochemically, the events regulating normal parturition. This consideration also is important to facilitate the development of suitable experimental models for preterm birth. We propose that the guinea pig fits these criteria better than currently used models and that this model should be further explored.

**Progestosterone concentrations in the guinea pig.** Perhaps the major advantage of the guinea pig is its similarity to the human regarding maternal serum progesterone concentrations in late gestation. As in the human, parturition in the guinea pig occurs when maternal progesterone concentrations are high and rising (Fig. 1) (35). The ovary is the major source of progesterone for the first 4 wk following conception, and then the placenta becomes predominant for the remainder of pregnancy (99). This luteolaplacental shift is remarkably similar to what occurs in human pregnancy (54, 56). In the guinea pig, luteal progesterone secretion appears to decline after days 35–40, and there is an accompanying significant fall in plasma progesterone concentration with the nadir of 75–200 ng/ml occurring at days 50–55 (35, 99, 180). Thereafter, there is some disagreement as to the pattern of progesterone concentrations. One study suggests placental progesterone production continues to increase, resulting in increasing maternal plasma concentrations for the next 2 wk until parturition occurs (35); others report no significant change in maternal progesterone concentrations in the final 2 wk of pregnancy (2, 99). Maternal plasma progesterone concentrations are ~150–300 ng/ml at parturition, similar to those in human pregnancy (19, 215). In contrast, maternal plasma progesterone concentrations in the rat reach peaks of 100–150 ng/ml at midpregnancy before falling to <10 ng/ml over the 2–3 days before parturition (14, 76). The pattern is similar in mice except that peak levels reach only ~80 ng/ml (219). In rabbits and sheep, maternal progesterone peaks at 10–20 ng/ml before falling rapidly in the days preceding parturition (16, 34, 76, 207).

In contrast to the rabbit and rat, large doses of progesterone or synthetic progestins failed to prolong pregnancy in the guinea pig (201, 231). Porter and colleague (166, 167) performed an extensive series of studies using a variety of doses, routes (intramuscular, intra-amniotic, intrauterine) and times of administration of progesterone or its pharmacological analogs and failed to detect any significant changes in uterine contractile activity or length of gestation. The conclusion was that progesterone is not a myometrial-blocking agent in the guinea pig.

In view of the lack of evidence for maternal serum progesterone withdrawal near term or any effect of exogenous progesterone on length of gestation in the guinea pig, the effects of administration of progesterone antagonists are of considerable interest. These studies have utilized mifepristone (RU-486), which has combined antiprogestin and antiglucocorticoid activity, as well as the more purely antiprogestin onapristone (ZK-98299). Removal of the corpora lutea before day 28 of guinea pig pregnancy often will result in abortion (99), similar to the human (55). In early human gestation (<35 days from conception), mifepristone alone is effective (65–85%) for induction of abortion, and this is considerably increased by addition of a uterine-stimulating prostaglandin (11, 218). At 43 ± 2 (SE) days of guinea pig gestation, treatment with antiprogestins interrupts only one-half of pregnancies, and the subsequent expulsion of the pups is protracted over several days. Similarly, at day 60 of gestation, most animals underwent parturition, but the labor often was protracted, suggesting that uterine contractions were not fully effective following the antiprogestin alone (46). However, at both time points, the antiprogestin treatment markedly enhanced the uterine responsiveness to OT or PGs. In summary, the response to antiprogestins appears to be quite similar in the guinea pig and human and quite distinct from that in rats.

As noted above, treatment of pregnant women or pregnant guinea pigs at midpregnancy (43 ± 2 days gestation) with an...
antiprogestin increased the sensitivity to OT (45). Surprisingly, the increased responsiveness to OT was not accompanied by an increase in OTRs, suggesting that in the guinea pig, these receptors are not inhibited by progesterone. This is in marked contrast to the situation in rats, where OTRs are rapidly increased following RU-486 treatment in mid-late pregnancy (66). The protracted course of the labor induced by antiprogestins during midpregnancy in either the human or guinea pig appears similar to the protracted labor that follows the use of OTR antagonists during labor at term in guinea pigs or rats (4, 197, 198). It also may be similar to the effects of OT antagonist given to women in preterm labor (187). These similarities between guinea pig and human support the notion that alterations in progesterone- and OT-dependent signaling events have separate but possibly consensual and modular actions in the process of parturition. Altered progesterone signaling may participate in cervical ripening, but a contractile stimulant such as OT is necessary to expel the products of conception in an efficient, physiological manner.

As discussed earlier, functional progesterone withdrawal may occur in primates, and this also may be present in the guinea pig (222). It also is important to consider that if progesterone signaling is important across all species, it is likely to be operative in concert with many other endocrine/paracrine/autocrine systems, including estrogen, CRH, and OT (205, 220).

**Gestational length in the guinea pig.** Goy et al. (89) carefully documented the natural progression of pregnancy in the guinea pig. In this species, the length of gestation is ~67 days with a standard deviation of 1.5 days. As in the human, the length of gestation and pup birth weight vary inversely with the number of fetuses. Approximately 7–8% of pregnancies in guinea pigs result in “preterm” birth of a live born pup that does not survive, and this mortality rate increases with litter size (131). These reproductive parameters are very reminiscent of the situation in the human, where the ~5% incidence of spontaneous (noniatrogenic) preterm birth (<37 wk of gestation) in singleton pregnancies increases with increasingly higher order multiple pregnancies (86). In contrast, as noted previously, spontaneous birth before fetal viability (hence “preterm”) is a very uncommon event in the animal models that have separate but possibly consensual and modular actions in the process of parturition. Altered progesterone signaling may participate in cervical ripening, but a contractile stimulant such as OT is necessary to expel the products of conception in an efficient, physiological manner.

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from other rodent models (32). These characteristics may render guinea pigs superior models for developmental and reproductive toxicology studies (179). This would make the model particularly attractive for testing any potentially therapeutic agents developed to prevent or arrest preterm labor.

**Perspectives and Significance**

There has been disappointingly little progress made over the last two decades toward understanding the mechanisms that result in spontaneous preterm birth. We have proposed that currently favored animal models have been, and will continue to be, useful in identifying potential regulatory mechanisms of parturition. However, these models are limited in a translational sense due to their reliance on parturition being precipitated by endocrine changes that are not shared with humans. We have reviewed the literature and suggest three major physiological considerations that should influence the decision on a more relevant animal model for human parturition. 1) It appears unlikely that a linear sequence of events leading to maternal progesterone withdrawal is the predominant factor in the timing of human parturition. This is a major deficiency in currently popular animal models (sheep, mice, rats, rabbits). 2) The concept of a simple “trigger” mechanism that initiates the sequence leading to progesterone withdrawal, derived from these models, is probably inappropriate for the human. We propose that human parturition occurs when several physiological systems (modules) synergize to transform the phenotype of the uterus from quiescent to contractile (MAPS). Preterm labor occurs when a critical number of modules are activated prematurely. 3) Labor is accompanied by a proinflammatory reaction of the immune system. There is an urgent need to determine whether, in the absence of intrauterine infection, this is merely an associative relationship or whether it is a cause-effect relationship. If the latter, it needs to be determined which is the cause and which is the effect.

We have reviewed the strengths and weaknesses of current animal models and suggest that the guinea pig comes closest to meeting these considerations. Although there are limited data regarding its mechanisms leading to parturition, these suggest remarkable similarity to the human. We also suggest that the guinea pig would be superior for other areas of research concerning the gestational influences on fetal growth and development. We believe that further characterization of this model is warranted and that the results may provide greater understanding of the mechanisms that regulate fetal development and the timing of birth. The principles of the 3Rs of Russell and Burch (replacement, reduction, and refinement) are gaining more attention across all fields of research (191). Selection of the best animal model to answer the particular biological question under consideration is a major principle of this philosophy. Although guinea pig physiology is not identical to that of humans, evidence favors it as the most appropriate model of parturition for translational impact to the human setting. Reinvestment in research using this model, in parallel with studies on human tissue, could enhance understanding of the mechanisms controlling parturition. This would provide a significant breakthrough in physiological knowledge and tremendous benefits to overall human health.

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**REFERENCES**


Review


