

A complex exchange of signals between mother and fetus determines when labor begins. By listening in, researchers hope to find ways to prevent or delay premature labor

Resetting Pregnancy's Clock

Premature babies look impossibly fragile. Their balled fists are as small as walnuts; their skin is translucent; tubes sprout from their bodies, connecting to devices that sustain life outside the womb.

In the United States, one out of eight babies is born prematurely—before 37 weeks instead of the usual 40—a rate that has increased 27% over the past 2 decades. Most preterm infants survive, many after a long stay in a neonatal intensive care unit, but they are at risk for disabilities such as mental retardation, cerebral palsy, lung and gastrointestinal problems, and vision and hearing loss. “The medical and nonmedical expenses associated with preterm birth probably exceed those of any other disease,” says Roberto Romero, a specialist in maternal-fetal medicine at the Detroit campus of the National Institute of Child Health and Human Development (NICHD).

For decades, researchers have had surprisingly few explanations for premature labor. Asymptomatic uterine infections, detected by the presence of microorganisms in the amniotic fluid, account for one-third to one-half of the cases, but the rest have no clear cause. As a result, preterm labor is very hard to predict, prevent, or stop.

But bright spots are emerging on the research landscape. Scientists have identified a human pregnancy clock powered by stress hormones that may be affected by diet and other factors. This could lead to early detection and perhaps prevention of some premature labor. Researchers are also unraveling the molecular events that unfold just before and during normal labor. Inflammation now appears to be central to the labor process, whether at term or preterm. Bacterial infections, allergic reactions, the stretching of uterine muscle, and the manufacture of a hormone from the fe-

tal lung can all spawn labor through inflammatory pathways, new research shows. Other data reveal mechanisms underlying the body's receptiveness to the pregnancy-prolonging hormone progesterone.

This cascade of findings is already pointing to potential targets for labor-stopping drugs that may eventually replace today's so-called tocolytics, which postpone labor for no longer than 48 hours and have potentially serious side effects. In the meantime, new methods for distinguishing true from false labor might ensure that tocolytics are given only to women who need them (see sidebar, p. 668).

These developments and the potential

in Dallas, “The mechanisms explaining how labor starts and progresses are finally starting to emerge.”

Like clockwork

The initiation of labor is driven by shifts in the levels of key hormones, chiefly estrogen and progesterone. During most of pregnancy, an abundance of progesterone, which is secreted by the placenta, relaxes the smooth muscle cells lining the uterus. It also keeps the ring of tissue at its bottom, the cervix, cinched tight with tough ropes of collagen, like the closure on a drawstring bag.

As the body prepares for labor, increasing amounts of estrogen, which opposes progesterone's actions, excite the uterine muscle. It also prompts the fetal membranes overlying the cervix to produce fatty acid hormones called prostaglandins. These soften the cervix by stimulating the production of enzymes that digest its collagen fibers. During labor, the uterus generates forceful contractions that eventually expel the fetus through the widened cervix.

In the mid-1990s, Smith and his colleagues unraveled a “placental clock” that appears to time the hormone shift. Levels of a placental

protein called corticotropin-releasing hormone (CRH), which promotes estrogen production, rise exponentially throughout pregnancy, they found. This rise is so predictable that they could use blood levels of CRH at 4 to 5 months of pregnancy to estimate when a woman would give birth.

“You can probably use CRH levels as a gauge of where you are in pregnancy,” says pediatrician Louis Muglia of Washington University in St. Louis, Missouri. The hormone might be used to identify women who will give birth early due to



Fragile beginnings. Endocrinologist Roger Smith with premature baby, born at 28 weeks (29 weeks in photo) at John Hunter Hospital in Australia.

payoffs are beginning to attract funds into a field that has long been short of cash. Last year, for example, the March of Dimes pledged \$75 million over 5 years to fight premature birth, in part through research into its causes. “It's a really exciting time,” says endocrinologist Roger Smith of the University of Newcastle in Australia. “There have been major changes in our understanding of preterm labor over the last couple of years.” Adds molecular endocrinologist Carole Mendelson of the University of Texas Southwestern Medical Center

accelerated placental clocks. Currently, the laboratory test for CRH is too cumbersome for routine clinical use, but researchers are working on alternatives.

Meanwhile, researchers are trying to determine what might make CRH build up to risky levels too early in a pregnancy. People have speculated about emotional stress—CRH is involved in the body's stress response—or genetic factors. So far, there has been little evidence to support these ideas. Last April, however, endocrinologist John Challis of the University of Toronto, Canada, and his colleagues reported data in sheep that may point to a different culprit: dieting.

Sheep put on a low-calorie diet from 60 days before until 30 days after conception had an extraordinarily high incidence of preterm birth, the team found: Half of them went into preterm labor. Calorie restriction would not have affected fetal growth, the Challis group reasoned, because 30-day fetuses require minimal nutrients. But the undernourished fetuses' pituitary and adrenal glands—which govern the stress response—matured prematurely. And the adrenal glands of the fetuses that were delivered early released unusually high levels of cortisol, a stress hormone whose release is driven by CRH. The cortisol surge, the researchers suggest, initiates labor, because it occurs in many species just before birth. Recent preliminary data further suggest that the placentas of undernourished sheep don't produce enough progesterone.

If the relation between lack of nourishment and early labor holds true for humans, Challis says, it "has enormous impact" for women trying to become pregnant. Dieting before pregnancy "may put them at higher risk of preterm labor," he says.

Even if eating well at the time of conception is important to an on-time delivery, many other factors can still intrude. So researchers are testing prophylactics that might prevent early labor by tipping the hormonal balance. At-risk women might be treated with a CRH antagonist such as antalarmin, which can delay delivery in sheep. But the most promising of the preventive measures so far is progesterone itself.

Obstetrician Paul Meis of Wake Forest University Baptist Medical Center in Winston-Salem, North Carolina, and his colleagues tested whether a metabolite of progesterone, 17 α -hydroxyprogesterone caproate, could prevent preterm labor in high-risk pregnant women. Weekly injections starting at 16 to 20 weeks reduced the rate of preterm delivery by 33% in 310 women compared to 153 women receiving a sham injection. The injections also reduced the incidence of certain complications, including some types of hemorrhage and need

for supplemental oxygen, in the newborns, the team reported last June.

Fighting fires

In many cases, however, it may not be possible to prevent the early onset of labor. It can start suddenly, for instance when an infection strikes. Various teams are trying to decipher the molecular events that take place once labor begins, in an effort to identify ways to halt it safely.

Many of the newly implicated molecules play roles in inflammatory pathways. In Au-

the uterus to contract. "We think NF- κ B is very central," says Bennett, whose group is now working on ways to inhibit its action.

What might cause the upsurge in NF- κ B during labor? Infection is one likely culprit. Bacterial toxins released during an infection, says Bennett, bind to receptors on macrophages or amniotic sac cells and activate NF- κ B through known signaling pathways.

Allergens may also incite labor through inflammatory pathways, according to work presented in February at the annual meeting of the Society for Maternal-Fetal Medicine. Pharmacologist Robert Garfield of the University of Texas Medical Branch (UTMB) in Galveston, NICHD's Romero, and their colleagues showed that injecting egg-white protein into pregnant guinea pigs that had been sensitized to it caused one-third of them to go into premature labor. By contrast, none of the nonsensitized guinea pigs or sensitized guinea pigs challenged with saline delivered prematurely. In addition, the scientists could prevent allergen-induced preterm birth by treating sensitized guinea pigs with a histamine-receptor antagonist, which blocks histamine-induced stimulation of uterine muscle. The data, according to the authors, "represent the first experimental evidence" that at least some types of allergic reactions can initiate preterm labor.

Closer to term, physically stretching the uterus may also provoke an inflammatory response through NF- κ B. Just before delivery, the head of the fetus usually presses down in the lower part of the uterus and stretches tissue there. Likely because of increased pressure, multiple fetuses raise the risk of prematurity, with the risk increasing incrementally with the number of babies in the uterus. Stretching the uterus in rats stimulates the expression of genes that code for labor-associated transcription factors and proteins, molecular biologist Stephen Lye of the Samuel Lunenfeld Research Institute in Toronto and his colleagues have found. Among those proteins, according to work reported earlier this year from Bennett's lab, is NF- κ B. Stretching activates NF- κ B in tissue from the human amniotic sac, although this effect was not found in uterine muscle.

The maturing fetus also prompts labor at term through a lung protein that activates inflammatory pathways, Mendelson and her team reported last month. The researchers detected this surfactant protein, dubbed SP-A,

Image not available for online use.

Inflammatory. Sonogram of twins. Multiple fetuses raise the risk of prematurity, perhaps because physically stretching the uterus provokes an inflammatory response that triggers labor. Bacterial infections, allergic reactions, and a hormone from the fetal lung can also spawn labor through inflammatory pathways.

gust 2003, Washington University's Muglia and his colleagues reported using DNA microarrays to measure changes in gene expression in human uterine tissues sampled either during labor or prior to labor. Many of the hundreds of genes whose expression was altered at labor coded for inflammatory proteins, and the pattern was surprisingly similar for both term and preterm samples.

A key event in this inflammatory response, according to work by obstetrician Phillip Bennett and his colleagues at Imperial College Hammersmith Hospital Campus in London, is the activation of the DNA-binding protein called nuclear factor κ B (NF- κ B), which triggers the transcription of inflammatory genes. In 2001, Bennett and his team discovered that NF- κ B revs up its gene transcription activity during labor, and in work presented last month at the annual meeting of the Society for Gynecologic Investigation they found that NF- κ B is also activated in preterm labor—at higher levels than in term labor.

Activation of NF- κ B, in turn, boosts production in the fetal membranes of the inflammatory cytokine interleukin-8 (IL-8) and the prostaglandin-producing enzyme cyclooxygenase 2 (COX-2), the Imperial College researchers have shown. Both IL-8 and prostaglandins soften the cervix and prepare

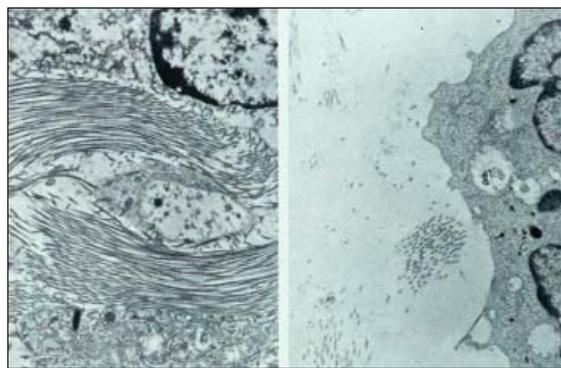
in the amniotic fluid of pregnant mice during the last 3 days of gestation. Levels of SP-A rose in parallel with similar rises in interleukin-1 and NF- κ B in macrophages present there. Cell culture work clarified that SP-A could stimulate the production of these inflammatory proteins by macrophages.

The UTMB researchers then discovered compelling evidence that this interaction can lead to labor: Injecting SP-A into the amniotic fluid of pregnant mice induced preterm delivery within 6 to 24 hours, whereas injecting an antibody to the surfactant protein or an NF- κ B inhibitor delayed labor by more than a day. The authors conclude that this fetal lung secretion “provides a key hormonal stimulus” for the inflammatory cascade in the uterus that leads to labor. “If we can understand how SP-A acts to induce labor at term, that opens up the possibility of finding therapeutic interventions for blocking preterm labor,” Mendelson says.

Recognizing the link to inflammation, some researchers suggest that anti-inflammatory drugs will prove useful in treating preterm labor. Newcastle’s Smith and his colleagues have recently launched a multicenter clinical trial of rofecoxib (Vioxx), which blocks the production of prostaglandins by inhibiting COX-2.

Stay calm

Aside from inflammation, a decline in responsiveness to progesterone is likely to be critical to the initiation of labor. Progesterone is thought to block genetic triggers of labor and maintain uterine quiescence. In species from rats to humans, blocking the receptor through which progesterone exerts its effects—the so-called B receptor—with a



Preparing the way. Cervical tissue from pregnant guinea pigs in midterm (*left*) and at the end of gestation (*right*). At term, enzymes degrade tough ropes of collagen (horizontal stripes in left image), softening the cervix.

drug such as RU486 will induce labor.

However, a drop in blood levels of progesterone does not accompany labor in humans, even though it does in nonprimates. Many researchers hypothesize that a “func-

Labor: True or False?

Only half of the women who show signs of preterm labor actually deliver early. In others, contractions die out on their own. But doctors have no reliable way to tell who needs potentially toxic drugs to stop contractions and who doesn’t. A device developed by pharmacologist Robert Garfield of the University of Texas Medical Branch in Galveston and his colleagues might change this.

In the 1990s, the researchers predicted the onset of labor in pregnant rats by monitoring the electrical activity in their uterine muscles. They found that the higher the frequency of electrical signals in the bursts that produce the muscle contractions, the closer a pregnant rat was to labor.

Last June, the researchers reported that the same holds true for pregnant women. They monitored the uterus’s electrical activity for 30 minutes in each of 99 pregnant women who were being checked at a clinic for either term or preterm labor. The team found a notable transition in the frequency spectrum at 24 hours before delivery in the term patients; in preterm patients, this occurred about 4 days before delivery, presumably because the labor process unfolds more slowly before term. The transition, Garfield says, can reveal with certainty when a patient is in true labor. The researchers are gearing up to test the device in a larger clinical trial, and they are working with medical instrument firm Fairway Medical Technologies in Houston to further develop the technology.

The same group has developed a device called a Collascope for measuring the softening of the cervix. A light beam excites the collagen in the cervix, causing it to glow at an intensity that is proportional to the tissue’s collagen content. That, in turn, reveals the proximity of labor, because collagen degrades as labor approaches. The researchers must still establish normal and abnormal profiles for the cervix’s collagen content during pregnancy, however.

A rapid assay of corticotropin-releasing hormone levels might also indicate the presence or imminence of labor (see main text).

—I.W.

tional” withdrawal of progesterone—that is, a decrease in the body’s receptiveness to it—initiates labor.

In June 2002, Newcastle’s Smith and his colleagues implicated a second progesterone receptor, dubbed A, that is believed to inhibit the B receptor. In uterine tissue samples from 24 pregnant women, the amount of messenger RNA for progesterone receptor A relative to that of receptor B was much higher during labor than prior to it. In February, Smith’s team went on to report that prostaglandins may be behind this effect, as they increase the ratio of A to B receptors in cultured human uterine muscle cells.

But other factors may also play a role in progesterone’s impotence during human labor. When progesterone binds to its receptor, the complex binds to DNA and promotes the transcription of genes. Several protein coactivators unwind the DNA so it can be transcribed. This past summer, Mendelson’s team reported that levels of three of these coactivators decrease in the uteruses of pregnant women and mice during labor. The researchers also found that giving pregnant mice a drug that keeps DNA unwound delayed labor for up to 2 days beyond the normal 19-day mouse pregnancy. The drop in the levels of coactivators, Mendelson infers, helps halt the expression of genes important

to keeping the uterus relaxed. NF- κ B also may contribute to functional progesterone withdrawal: Data from the mid-1990s suggest that it represses the activity of the progesterone receptor by directly binding to it.

While many groups are probing the molecular signals that induce labor, others are looking for inherited genetic variations associated with preterm labor. In 2002, a team led by obstetric anesthesiologist Richard Smiley of Columbia University in New York City identified a polymorphism in the β_2 adrenergic receptor, which relaxes uterine smooth muscle, that lowers the risk of preterm delivery among Hispanic women, probably by maintaining the receptor’s sensitivity to repeated stimulation.

Now Muglia and his colleagues are starting a 5- to 10-year search for polymorphisms that elevate the risk of preterm delivery among a broader population of women. The researchers will comb the genomes of families with a strong history of premature labor. They will also compare the genomes of women who have had a premature delivery with those of women who have had full-term pregnancies. “It would give us great insight into the mechanism if we could identify just one gene involved,” Muglia says.

“I’m optimistic that in 5 years we will fit the different pieces of the puzzle together and that will guide us to a way to intervene,” Smith says. Meanwhile, he adds, the puzzle remains only partly assembled: “We still don’t understand how we get born.”

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