Mammalian Labor: Variations on a Theme by Amniota

Roger Smith, Tamas Zakar, and Gemma Madsen

Mothers and Babies Research Centre, Hunter Medical Research Institute, University of Newcastle, Newcastle 2310, Australia

In this month's issue of *Endocrinology*, Wada et al (1) report on the "Role of vascular endothelial growth factor (VEGF) in maintenance of pregnancy in mice." It's a beautifully written report of a carefully performed series of experiments that strongly implicate a fall in the corpus luteum production of VEGF in the onset of labor in mice. However, the most intriguing and insightful aspect is the focus within the title on pregnancy maintenance.

Maintaining the fertilized egg within the reproductive tract is a key issue for all amniotes. Before the evolution of the amniote egg, most vertebrate females laid their eggs in water and fertilization was achieved by the male depositing sperm in the vicinity (2). The ability to be fully terrestrial required the evolution of the amniote egg that was covered with membranes that were impervious to water so they did not dry out, yet permeable to oxygen and carbon dioxide allowing respiration. However, the membranes and shell of the amniote egg are formed inside the reproductive tract and the formed coverings are impermeable not only to water but also to sperm. The physiological consequence of the internal creation of the egg coverings is the need also for internal fertilization before the formation of the impenetrable membranes.

Movement of the ovum from the ovary to the exterior down the reproductive tract is seen in fish and amphibians in anticipation of external fertilization. This transport is effected by both peristaltic contraction and cilial motion (3). In contrast, for all amniotes, a central requirement is a female reproductive tract that allows the penetration of the sperm against the usual peristaltic and cilial transport toward the exterior. Once fertilization has occurred within the reproductive tract of the amniote, the fertilized ovum must be retained to allow formation of the membranes and in some species the shell or outer covering.

A critical evolutionary step for the amniote is the development of a mechanism to retain the zygote within the reproductive tract. Although direct studies of early amniotes are impossible, the physiology of extant members of the group may provide clues. Extant amniotes include birds, reptiles, and mammals. In these diverse classes of vertebrates, the endocrinological processes that arrest contractions of the reproductive tract or uterus have been examined. In the chicken, progesterone plays a key role in retaining the egg within the oviduct. Equally, in the lizard and other reptiles, corpus luteum production of progesterone is critical (4). Within mammalia, the role of progesterone has been examined in many species. In the sheep, murides, marsupials (5), and in humans, progesterone withdrawal is associated with the onset of labor indicating a role for progesterone in reducing contractile activity of the myometrium. Therefore, progesterone as an agent for the inhibition of uterine or reproductive tract activity is found in members of all extant classes of amniotes. This is evidence that progesterone was used to maintain reproductive tract quiescence in the ancestral root amniote. This view is also supported by genome comparison studies that support the evolution of the progesterone receptor (PR) before the evolution of amniotes (6).

The data indicate that progesterone allows the amniote to delay the passage of the zygote through the reproductive passage to permit additional parental investment. In some amniotes, the investment of the mother is in the form of egg yolk and the formation of a shell. In placental mammals, retention of the zygote allows additional development of the embryo and fetal growth before release into the exter-

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Abbreviations: ER, estrogen receptor; miR, micro-RNA; PR, progesterone receptor; VEGF, vascular endothelial growth factor; ZEB1, 2, zinc finger E-box-binding homeobox 1, 2.

nal environment. To release the developed conceptus, the relaxing actions of progesterone must be counteracted or blocked in some way. The physiological brake on uterine contractility created by progesterone must be removed to allow birth. The onset of uterine contractions and subsequent birth is therefore not so much a turning on of contractions but a removal of the brake on contractions as suggested many years ago by Csapo (7).

Mammals invest considerably in their offspring before birth. Despite this additional investment to create a larger and more developed offspring, neonatal mortality is high in mammals. In some species, the physical size of the offspring is such that obstruction of the fetus in the pelvis of the mother can be a cause of maternal death as in cattle (8), horses (9), and humans (10). The most appropriate time for birth and the most appropriate length of gestation is therefore a trait on which evolution will act powerfully.

Gestational length varies enormously among different species of mammal. Some species such as mice and cats deliver altricial or immature young requiring diligent maternal care for an extended period of time before they become independent. Other species such as horses and elephants have longer periods of gestation and deliver relatively mature offspring able to move with the herd soon after birth. These diverse strategies represent the different life histories of the species. In general, gestational length is related to the size of the mother, the number of the offspring, and intriguingly, the size of the neonatal brain. This relationship has been described by the equation $G_{calc} = 26L^{-0.6} E^{0.06} P_a{}^{0.18}$ where G_{calc} = calculated gestational length, L = litter size, E = neonatal brain weight, and P_a = weight of the nonpregnant adult female (11). Although this equation tightly predicts gestation length in a wide variety of mammals from elephants to shrews it does not explain the underlying physiology.

With such marked evolutionary pressure on preparing a conceptus for survival as a neonate, a range of mechanisms have evolved to optimize the timing of birth. The force of this evolutionary pressure is evidenced by our method of categorization of distinct species on the basis of whether interbreeding leads to viable fertile offspring. Distinct species are therefore defined by their differences in reproduction. The evolutionary pressure generated by high rates of perinatal death has led to remarkable variations in the mechanisms that determine progesterone withdrawal at the time of labor.

In a large number of species, circulating maternal plasma concentrations of progesterone fall dramatically before the onset of uterine contractions. Examples of species in which circulating maternal progesterone concentrations fall to induce the onset of labor include sheep and goats, related even-toed ungulates. The apparent similarity is a mirage because the fall in circulating progesterone in the goat is due to maternal luteolysis (12), whereas in the sheep, progesterone production drops due to changes in placental steroidogenesis (13). However, in some species, the circulating concentrations of progesterone do not fall and progesterone withdrawal to allow uterine contractions appears to be functional; that is, some mechanism interferes with the action of progesterone.

Guinea pigs are one example of a species where progesterone concentrations do not fall before labor (14); humans are another (15). In the human, progesterone concentrations have been carefully examined across gestation. Plasma levels of progesterone begin rising just before ovulation from ovarian production, and this increases with the formation of the corpus luteum. If a pregnancy occurs, levels continue to rise in an almost linear pattern throughout gestation, although the predominant site of production changes from the ovary to the placenta at about 7 to 10 weeks of gestation (16). At the end of pregnancy, there is some suggestion of a flattening in the rate of rise but little evidence of any dramatic decline as seen in so many other mammals. However, there is evidence of a functional withdrawal of progesterone action. Notably, genes that are normally repressed by progesterone are increased at the end of human pregnancy in association with the onset of labor (17). Important among such genes are the estrogen receptor (ER)α which is the predominant form of the ER expressed in the myometrium (18). This process of functional withdrawal has been explored by a number of investigators who have explored several different hypotheses.

Hypotheses for the functional withdrawal of progesterone action include metabolism of progesterone (19), endogenous progesterone antagonism (20), loss of coactivators required for transactivation of the progesterone-sensitive genes (21) and alterations in expression of PRs (17). Recent evolution in the PR within primates may provide a biochemical basis for the changes in progesterone withdrawal mechanisms in humans (22). There are two major forms of the PR, which are created by expression from two different promoters within the single gene (23). PR-B is the principal activator of progesterone-responsive genes, whereas PR-A can repress these actions of PR-B. Therefore, as the PR-A/PR-B ratio increases, target tissue responsiveness to progesterone decreases, consistent with a functional withdrawal of progesterone. Several groups have demonstrated that the expression and protein levels of the PR-A isoforms are increased in the human myometrium at the time of labor compared with term nonlaboring tissues (17, 24). In vitro studies showed that prostaglandins increased the PR-A/PR-B expression ratio in an immortalized pregnant human myometrial cell

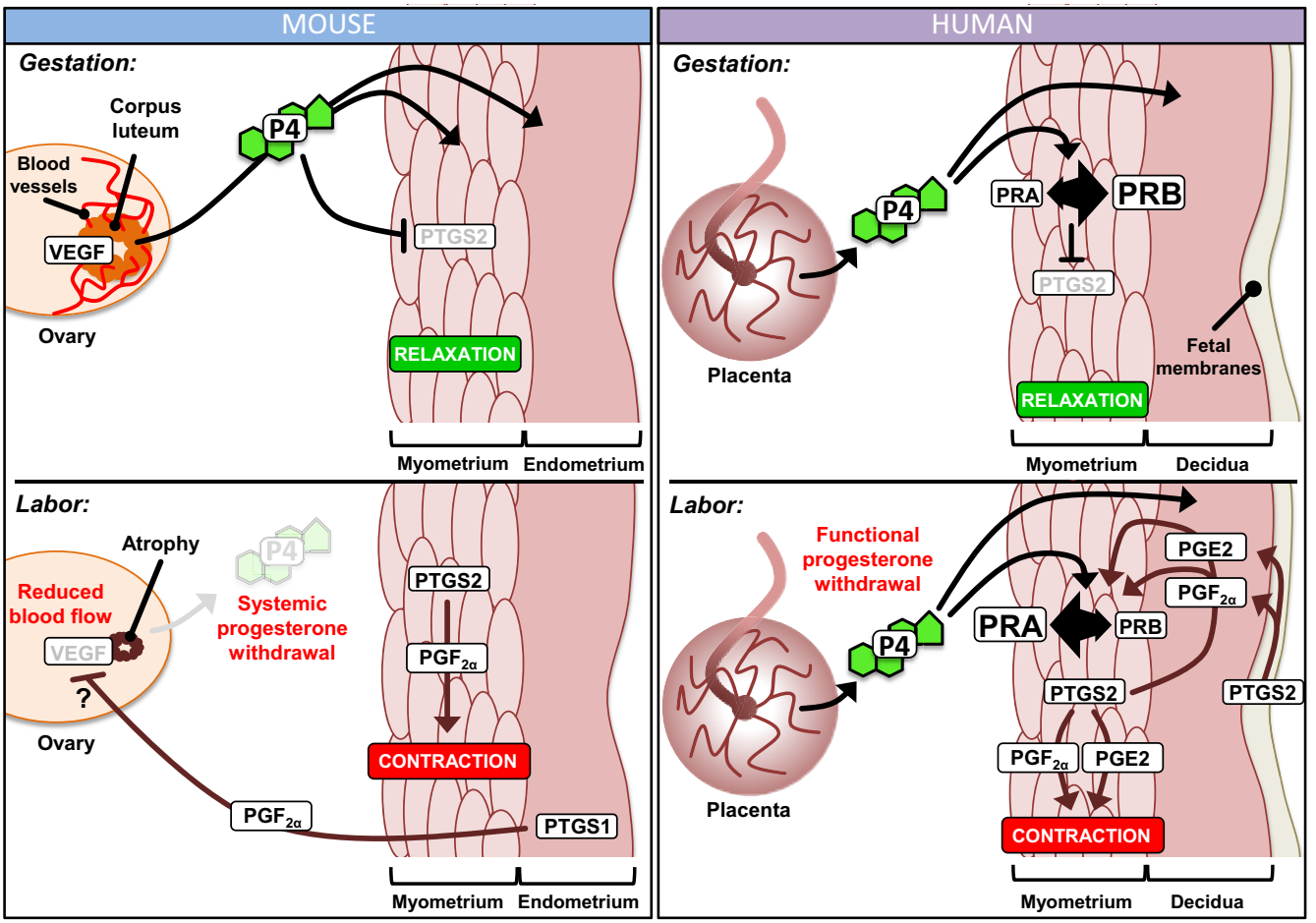

Figure 1. Analogous role of prostaglandins in progesterone withdrawal before birth in the mouse and the human. In late gestation progesterone (P4) is secreted by the corpus luteum in the mouse and by the placenta in the human. Progesterone suppresses contraction-associated protein expression and prostaglandin production in the myometrium in both species. In the mouse, prostaglandin F2α (PGF2α) produced in the endometrium by prostaglandin synthase (PTGS)-1 causes atrophy of the corpus luteum possibly by depressing the VEGF system, which results in systemic progesterone withdrawal. In the human, PGF2α and PGE2 produced by PTGS2 in the fetal membranes modulate PR isoform ratio in the myometrium toward PRA dominance, decreasing progesterone responsiveness and causing functional progesterone withdrawal. Both types of progesterone withdrawal result in PTGS2 induction and the generation of labor-promoting prostaglandins in the myometrium.

line, PHM1-31, by selectively increasing PR-A mRNA levels (25).

Most recently, Carole Mendelson's laboratory has provided evidence supporting the involvement of microRNAs (miRs) in mediating progesterone action in the pregnant myometrium (26–28). A key finding of these studies is that PR-bound progesterone up-regulates the negative transcriptional regulator *ZEB1* (zinc finger E-box-binding homeobox 1) in myometrial cells. ZEB1 inhibits the expression of the contraction-associated proteins oxytocin receptor and connexin 43 and down-regulates the miR-200 family, which inhibits in a reciprocal fashion ZEB1 and the homologous ZEB2. Furthermore, the miR200 family also targets the transcription factor STAT5b (signal transducer and activator of transcription 5B), which blocks the myometrial expression of the progesterone-metabolizing enzyme 20α-hydroxysteroid dehydrogenase. In addition, ZEB1 up-regulates the miR-199a-3p/214 family, which targets prostaglandin synthase-2, the limiting enzyme of prostaglandin biosynthesis. Myometrial progesterone responsiveness is high during pregnancy, which shifts this system toward low contraction-associated gene expression, low prostaglandin production, and low progesterone metabolism. Progesterone action is withdrawn at term, which readjusts the state, resulting in an increase of contraction-associated protein levels, prostaglandin production, and local progesterone metabolism, all promoting the onset of labor. These new findings highlight the pivotal importance of the mechanisms that control myometrial progesterone responsiveness during pregnancy. Changing PR isoform expression appears critical in this regard (17, 29), and recent work has suggested that prostaglandins may be involved in *PR* gene regulation via transcriptional and possibly epigenetic pathways (25). Chai et al (30) reported that the activating histone modification H3K4me3 increased at

both *PR* promoters with labor, but the increase was significantly higher at *PR-A* compared with *PR-B*.

These studies introduce a role for prostaglandins into the process of progesterone withdrawal. Prostaglandins are strongly implicated in the processes of labor across the order mammalia. Even in the marsupial macropods such as the tammar wallaby, injections of prostaglandin will produce parturient behavior in nonpregnant females (31). In many species including humans prostaglandins can be used to induce labor. The exact way in which prostaglandins are physiologically involved in human parturition remains unclear although evidence exists that myometrial interactions between the key prostaglandin synthetic enzyme prostaglandin synthase-2, ERα, and the PRs are robust before labor and change with the onset of labor (18). (Potential interactions between prostaglandins and progesterone in human parturition are contrasted with those in the mouse in Figure 1.)

Returning to the recent study by Wada et al (1), the work on the mouse implicates VEGF as a critical factor in producing the fall in circulating maternal progesterone concentrations that precipitate the onset of uterine contractions and labor in this species. VEGF acting through its receptor is shown to be responsible for the increase in corpus luteum blood flow. The drop in VEGF before labor is shown to reduce blood flow and promote fibrin deposition in blood vessels, leading to reduced progesterone production although basal levels of progesterone synthesis by luteal cells remain unchanged. The most interesting results lie in the future when the role of prostaglandins in regulating the ovarian VEGF system can be tested. It seems probable that in the mouse, the variation on the amniote theme of progesterone arrest of myometrial contraction is terminated by prostaglandins working through VEGF signaling in the corpus luteum.

The relevance of the findings by Wada et al (1) to human parturition is presently unclear. Human pregnancy does not depend on a functioning corpus luteum after the first trimester, because the main supplier of progesterone is the placenta after the luteo-placental shift. Nevertheless, VEGF is produced by human gestational tissues and production increases with term spontaneous labor (32). Using an animal model that exhibits a luteo-placental shift of progesterone production and delivery in the presence of persistently high maternal progesterone levels may be one way of exploring the role of angiogenic factors like VEGF in the parturition process with more relevance to the human. The guinea pig is a candidate for such studies, because it meets these criteria and undergoes functional progesterone withdrawal before birth that involves a reduction of myometrial PR expression (33). Prostaglandins induce delivery in guinea pigs like in other mammals, but the involvement of VEGF in guinea pig parturition remains to be explored in the future. The extreme diversity of mechanisms regulating the onset of labor in different mammals makes direct extrapolation of animal studies to humans inappropriate, but comparative physiological studies such as those of Wada et al (1) can identify new candidates for exploration in human clinical studies.

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Address all correspondence and requests for reprints to: Roger Smith, Mothers and Babies Research Centre, Hunter Medical Research Institute, University of Newcastle, Newcastle 2310, Australia.

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References

1. **Wada et al.** Role of vascular endothelial growth factor (VEGF) in maintenance of pregnancy in mice. *Endocrinology*. 2013;154:900–910
2. **Colbert EH, Morales M, Minkoff EC.** *Colbert's Evolution of the Vertebrates: A History of the Backboned Animals Through Time*. 5th ed. New York, NY: John Wiley, Sons; 2001
3. **van Tienhoven A.** *Reproductive Physiology of Vertebrates*. Philadelphia, PA: Saunders; 1968
4. **Guillette A.** Viviparity in reptiles: evolution and reproductive endocrinology. In: Norris DO, ed. *Hormones and Reproduction of Vertebrates*. San Diego, CA: Academic Press; 2011
5. **Renfree MB, Blanden DR.** Progesterone and oestrogen receptors in the female genital tract throughout pregnancy in tammar wallabies. *J Reprod Fertil*. 2000;119:121–128.
6. **Eick GN, Thornton JW.** Evolution of steroid receptors from an estrogen-sensitive ancestral receptor. *Mol Cell Endocrinol*. 2011; 334:31–38.
7. **Csapo A.** Progesterone "block". *Am J Anat*. 1956;98:273–292.
8. **Mee JF, Berry DP, Cromie AR.** Risk factors for calving assistance and dystocia in pasture-based Holstein-Friesian heifers and cows in Ireland. *Vet J*. 2011;187:189–194.
9. **Freeman DE, Hungerford LL, Schaeffer D, et al.** Caesarean section and other methods for assisted delivery: comparison of effects on mare mortality and complications. *Equine Vet J*. 1999;31:203–207.
10. **Kadowa I.** Ruptured uterus in rural Uganda: prevalence, predisposing factors and outcomes. *Singapore Med J*. 2010;51:35–38.
11. **Hofman MA.** Evolution of brain size in neonatal and adult placental mammals: a theoretical approach. *J Theor Biol*. 1983;105:317–332.
12. **Ford MM, Young IR, Caddy DJ, Thorburn GD.** Fetal and maternal endocrine changes approaching parturition in the goat: lack of evidence for prostaglandins E2 and F2alpha as signals for luteolysis. *Biol Reprod*. 1998;58:1065–1070.
13. **Liggins GC, Fairclough RJ, Grieves SA, Forster CS, Knox BS.** Parturition in the sheep. *Ciba Found Symp*. 1977;47:5–30.
14. **Illingworth DV, Challis JR, Henville A.** Plasma levels of progesterone and oestrogen in the pregnant guinea-pig. *J Reprod Fertil*. 1971; 25:305–306.
15. **Smith R, Smith JI, Shen X, et al.** Patterns of plasma corticotropin-releasing hormone, progesterone, estradiol, and estriol change and

the onset of human labor. *J Clin Endocrinol Metab*. 2009;94:2066–2074.
16. **Csapo AI, Pulkkinen MO, Ruttner B, Sauvage JP, Wiest WG.** The significance of the human corpus luteum in pregnancy maintenance. I. Preliminary studies. *Am J Obstet Gynecol*. 1972;112:1061–1067.
17. **Mesiano S, Chan EC, Fitter JT, Kwek K, Yeo G, Smith R.** Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. *J Clin Endocrinol Metab*. 2002;87:2924–2930.
18. **Bisits AM, Smith R, Mesiano S, et al.** Inflammatory aetiology of human myometrial activation tested using directed graphs. *PLoS Comput Biol*. 2005;1:132–136.
19. **Sheehan PM, Rice GE, Moses EK, Brennecke SP.** 5 Beta-dihydroprogesterone and steroid 5 beta-reductase decrease in association with human parturition at term. *Mol Hum Reprod*. 2005;11:495–501.
20. **Allport VC, Pieber D, Slater DM, Newton R, White JO, Bennett PR.** Human labour is associated with nuclear factor-κB activity which mediates cyclo-oxygenase-2 expression and is involved with the 'functional progesterone withdrawal'. *Mol Hum Reprod*. 2001;7:581–586.
21. **Condon JC, Jeyasuria P, Faust JM, Wilson JW, Mendelson CR.** A decline in the levels of progesterone receptor coactivators in the pregnant uterus at term may antagonize progesterone receptor function and contribute to the initiation of parturition. *Proc Natl Acad Sci U S A*. 2003;100:9518–9523.
22. **Chen C, Opazo JC, Erez O, et al.** The human progesterone receptor shows evidence of adaptive evolution associated with its ability to act as a transcription factor. *Mol Phylogenet Evol*. 2008;47:637–649.
23. **Giangrande PH, Pollio G, McDonnell DP.** Mapping and characterization of the functional domains responsible for the differential activity of the A and B isoforms of the human progesterone receptor. *J Biol Chem*. 1997;272:32889–32900.
24. **Pieber D, Allport VC, Hills F, Johnson M, Bennett PR.** Interactions between progesterone receptor isoforms in myometrial cells in human labour. *Mol Hum Reprod*. 2001;7:875–879.
25. **Madsen G, Zakar T, Ku CY, Sanborn BM, Smith R, Mesiano S.** Prostaglandins differentially modulate progesterone receptor-A and -B expression in human myometrial cells: evidence for prostaglandin-induced functional progesterone withdrawal. *J Clin Endocrinol Metab*. 2004;89:1010–1013.
26. **Renthal NE, Chen CC, Williams KC, Gerard RD, Prange-Kiel J, Mendelson CR.** miR-200 family and targets, ZEB1 and ZEB2, modulate uterine quiescence and contractility during pregnancy and labor. *Proc Natl Acad Sci U S A*. 2010;107:20828–20833
27. **Williams KC, Renthal NE, Condon JC, Gerard RD, Mendelson CR.** MicroRNA-200a serves a key role in the decline of progesterone receptor function leading to term and preterm labor. *Proc Natl Acad Sci U S A*. 2012;109:7529–7534.
28. **Williams KC, Renthal NE, Gerard RD, Mendelson CR.** The microRNA (miR)-199a/214 cluster mediates opposing effects of progesterone and estrogen on uterine contractility during pregnancy and labor. *Mol Endocrinol*. 2012;26:1857–1867
29. **Merlino AA, Welsh TN, Tan H, et al.** Nuclear progesterone receptors in the human pregnancy myometrium: evidence that parturition involves functional progesterone withdrawal mediated by increased expression of progesterone receptor-A. *J Clin Endocrinol Metab*. 2007;92:1927–1933.
30. **Chai SY, Smith R, Zakar T, Mitchell C, Madsen G.** Term myometrium is characterized by increased activating epigenetic modifications at the progesterone receptor-A promoter. *Mol Hum Reprod*. 2012;18:401–409.
31. **Hinds LA, Tyndale-Biscoe CH, Shaw G, Fletcher TP, Renfree MB.** Effects of prostaglandin and prolactin on luteolysis and parturient behaviour in the non-pregnant tammar, *Macropus eugenii*. *J Reprod Fertil*. 1990;88:323–333.
32. **Marvin KW, Keelan JA, Eykholt RL, Sato TA, Mitchell MD.** Expression of angiogenic and neurotrophic factors in the human amnion and choriodecidua. *Am J Obstet Gynecol*. 2002;187:728–734.
33. **Welsh T, Caritis S, Hirst J, Mesiano S, Zakar T.** Progesterone withdrawal in the pregnant guinea pig is mediated by decreased uterine PR expression and stimulated by prostaglandin in vivo. *Reprod Sci*. 2012;19 (Suppl 3):192A