Steroidogenesis and the initiation of parturition

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Overview

One of the most fundamental axioms of mammalian reproduction is that pregnancy requires the support of progesterone without which it cannot be established or maintained. Though this basic physiological tenet was accepted long ago, major gaps in our understanding of the physiology of both pregnancy and parturition remain which hamper our ability to solve clinically and agriculturally significant problems such as low fertility, fetal growth restriction, preterm birth and poor neonatal outcomes. The historical reliance of our understanding of both pregnancy and parturition on this single hormone, and how it has been measured in the vast majority of studies, may represent a tangible weakness and impediment to progress. Other weaknesses include a desire to fit all species into a unified paradigm, and a reluctance to accept that physiological processes regulated by progesterone or other progestins in different tissues might vary in reliance on classic (nuclear receptor) versus other, non-classical mechanisms of action. The relative importance of these distinct response pathways in certain cells or tissues also may differ across species, as does so much of basic reproductive physiology. It is well known that certain species are reliant on luteal function throughout gestation, whereas the placenta subsumes endocrine support in others (Geisert \& Conley 1998), yet progesterone alone is still believed to be the single common element. As radical as it might seem, however, progesterone may not be the single common hormone of pregnancy in mammals.

Combine these caveats with the fact that only a relatively small number of the 5,500 or so species of mammals have been evaluated throughout pregnancy, and it seems clear that our understanding of the role of steroids in pregnancy and parturition is poor at best. In this review we will address steroidogenesis and the events that bring about parturition, but will do so in an attempt to highlight potential weaknesses in the commonly held assumptions that have become the basis for designing and interpreting studies on the maintenance of pregnancy and the initiation of parturition in domestic species. We would like to think that the pioneers of steroid biology would welcome such a discussion, and might even wonder with all the studies conducted, and/or modern methodologies applied, why we have made so little progress in addressing this fundamental question.
A discussion of the endocrine control of pregnancy and parturition seems incomplete without reflecting on the history of how progesterone was identified and its role defined, because much of our current understanding is founded on assumptions derived from those foundational discoveries. If it was Berthold and Brown-Sequard that gave life to the notion of internal secretions, it was the work of many others that began to clarify the complexity of the sex steroid hormones and their actions. As Marshall put it in 1922, almost a century ago, “We are thus forced to conclude that the phenomena of pregnancy and parturition are brought about by chemical stimuli acting through the blood-stream (Marshall 1922).” This basic understanding of pregnancy and parturition were crucial in establishing the existence of the progestogenic class of steroid hormones, specifically as it relates to the endocrine function of corpora lutea and the placenta. The effects of progestins on the endometrium led ultimately to the chemical identification of progesterone itself. Specifically, isolation of bioactive extracts from corpora lutea, was followed by the purification, crystallization and eventually the determination of the formula of progesterone, all of which was accomplished through the combined efforts of several groups between 1929-1934 using in vivo bioassays evaluating endometrial responses (Corner 1946).

In fact, several bioassays were developed and used during that period. Most, like the Corner-Allen assay which used adult female rabbits (Corner & Allen 1929), or the Clauberg-McPhail assay in immature, estrogen-primed rabbit does (McPhail 1934), assessed the degree of endometrial proliferation or decidual cell response (Astwood 1939) induced by compounds administered by injection (Glasser 1975). Studies on the relative potencies of endogenous pregnanes were few. Instead, the search for synthetic, orally active progestins refocused investigators toward developing therapeutic compounds. However, as some have pointed out, the most reliable and relevant bioassay is the maintenance of pregnancy, and many of the synthetic progestins that stimulate endometrial development may not be capable of doing so (Glasser 1975). More importantly, few endogenously synthesized pregnanes (putative progestins) have been tested for their ability to sustain pregnancy. Studies such as these are particularly difficult to conduct in large animal species not only because of the doses required but also because of the relatively long gestation length. Therefore, in contrast to the multiple steroids populating the estrogen, androgen and corticoid classes, after eight decades the progestin class still contains only a single, physiological steroid, progesterone, whose bioactivity had been definitively established.

The focus on progesterone as the sole physiological progestin, and the potential existence of other bioactive progestins

How plausible is it that progesterone is the only physiological progestin in mammals? As early as 1959, Short commented that circulating progesterone concentrations in pregnant mares were surprisingly low, <4 ng/ml (Short 1959). This landmark observation was confirmed and extended decades later by Holtan et al. (Holtan et al. 1975; Holtan et al. 1991) and others (Ousey et al. 2005), who showed, ultimately using gas chromatography mass spectrometry, that progesterone concentrations in mid to late equine gestation were <0.5ng/ml, including in the circulation of the fetal foal (Ousey et al. 2003). Conversely, circulating concentrations of 5α-reduced metabolites like 5α-dihydroprogesterone (DHP) were very high (Hamon et al. 1991; Holtan et al. 1991; Ousey et al. 2003). Moreover, horses are not unique in this regard – very low to undetectable plasma concentrations of progesterone are found in zebras (Klima et al. 1999), elephants (Hodges et al. 1997) and the rock hyrax (Kirkman et al. 2001).
Importantly, DHP has also been shown to compete equally with progesterone for binding using equine endometrial (Jewgenow & Meyer 1998) and mammary (Chavatte-Palmer et al. 2000) extracts, endometrial extracts of the elephant (Meyer et al. 1997; Jewgenow & Meyer 1998; Greyling et al. 1997) and rock hyrax (Kirkman et al. 2001), and to lesser, more variable degrees in other species (Jewgenow & Meyer 1998). Unfortunately, attempts to demonstrate bioactivity of DHP on equine myometrial contractility were unsuccessful, but progesterone was no more active in those assays (Ousey et al. 2000). Thus, for some time alternative or additional endogenous pregnanes with progestational activity have been postulated to exist but never confirmed. Consequently, studies on pregnancy and parturition have remained focused on measuring progesterone, most without some form of chromatography and using immunoassays with primary antisera that necessarily cross-react (Behrman 1988) with multiple pregnanes of unknown bioactivity and therefore unknown significance.

Recent efforts in one of our laboratories have attempted to build on the pioneering studies in equine pregnancy cited above (Short 1959; Holtan et al. 1975; Holtan et al. 1991; Jewgenow & Meyer 1998; Meyer et al. 1997), by re-examining the bioactivity of DHP in vivo and in vitro (Scholtz et al. 2014). We first demonstrated that DHP can induce equine endometrial growth and profoundly stimulate endometrial expression of the progesterone-responsive genes uterocalin (Crossett et al. 1996; Crossett et al. 1998) and uteroglobin (Muller-Schottle et al. 2002; Beier-Hellwig et al. 1995) in ovariectomized mares (Scholtz et al. 2014). Interestingly, Kontula et al. also reported in rabbits that DHP was highly uterotropic if administered locally into the uterus (Kontula et al. 1975), but not if administered by injection (Rahman et al. 1975). Second, we showed that DHP (but not vehicle) could maintain equine pregnancies with normal fetal development to day 27 after progesterone was withdrawn by inducing luteal regression on day 14 (Scholtz et al. 2014). Third, we demonstrated using a reporter assay in vitro [MMTV-luciferase in HepG2 cells, co-transfected with expression constructs encoding either equine or human progesterone receptor (PR)] that DHP activates the equine PR with equal potency and efficacy to progesterone itself and does so at concentrations seen during the luteal phase and second half of equine gestation (Scholtz et al. 2014). Although exhibiting one-fifth the biopotency of progesterone in terms of activating the human PR in our studies (Scholtz et al. 2014), DHP exhibits high affinity binding to human myometrial extracts (Kontula et al. 1975), and was equally efficacious in our in vitro bioassay at concentrations found circulating systemically in women in their third trimester (Milewich et al. 1975; Hill et al. 2007). The binding of DHP to the ligand-binding domain differs among the PR of various species, with binding of DHP to endometrial cytosolic extracts from elephants and horses equal to or greater than that for progesterone (Wierer et al. 2012), consistent with our in vitro bioactivity data (Scholtz et al. 2014). This is an important, if simple, concept. Steroids do not themselves evolve, and as conserved as their actions may be across species, the steroid receptors do and have evolved to respond to different endogenous agonists (Baker 2001; Baker 1997; Baker & Uh 2012). Thus, we have answered our question whether progesterone is the only potent, physiological agonist in the progestin class, and indeed it is not. It seems to us equally likely that DHP is not the only endogenous progestin in mammals, even in species like the horse where DHP exhibits bioactivity comparable to progesterone (Scholtz et al. 2014). The challenge then is to define relative biopotencies of DHP and various other potential progestins across species using species-specific bioassays and measuring endogenous concentrations by appropriately specific methods.

Refocusing attention on DHP as a bioactive progestin of physiological significance, in some species at least, immediately implicates the 5α-reductase enzyme system as important in the physiology of pregnancy and perhaps even the initiation of parturition. Evidence suggests that
of the two major isoforms, the 5α-reductase type 1 isozyme is likely to play the more important role in conversion of progesterone to DHP than is the type 2 isozyme, and our studies in horses support this. There was a clear predominance of type 1 over type 2 expression in equine endometrium and chorio-allantois from late pregnancy based on quantitative transcript analysis (Scholtz et al. 2014). Expression of the type 1 isozyme predominates in human (Milewich et al. 1979) and mouse placenta (Mahendroo et al. 1996) also, and in human corpora lutea as well (Haning, Jr. et al. 1996). Moreover, since women with 5α-reductase type 2 deficiency have normal concentrations of DHP (Milewich et al. 1995), 5α-reductase type 1 isozyme alone is adequate and likely responsible for the synthesis of DHP. In contrast, sheep placentas at term appear to exhibit higher levels of expression of the 5α-reductase type 2 than type 1 based on transcript analysis. Still, there is much greater expression of 5α-reductase in caruncles (maternal) than in cotyledons (fetal placental; Fig. 1).

![Graph showing expression of 5α-reductase isozymes 1 and 2 in sheep placenta at term](image)

**Fig. 1.** Expression (qPCR) of mRNA for 5α-reductase isozymes 1 and 2 in sheep placenta at term (149.6 ± 0.5 days). CAR = caruncle = maternal placenta; COT = cotyledon = fetal placenta. Two of the ewes had twin lambs; the remainder had singletons. LP Reynolds, AJ Conley, CO Lemley, KA Vonnahme, & JS Caton (unpublished observations).

No known cases of 5α-reductase type 1 deficiency have yet been found in nature (Griffin et al. 2001), even though there are many reported deficiencies of the type 2 isozyme in humans (Imperato-McGinley & Zhu 2002; Wilson 2001; Wilson et al. 1993) and some likely in horses
Steroidogenesis and the initiation of parturition (Knobbe et al. 2011). However, the role of 5α-reductase type 1 in pregnancy is suggested by the results of gene knockouts in mice which induces fetal death (Mahendroo et al. 1997) and impairs cervical ripening thereby preventing normal delivery (Mahendroo et al. 1999). In addition, in all species studied and reported to date, both isozymes metabolize progesterone more efficiently than testosterone (Wilson 1975; Russell & Wilson 1994; Levy et al. 1995), suggesting that the 5α-reductase type 1 and 2 isozymes are more adapted for progesterone metabolism than for testosterone. These observations are consistent with the notion that the physiological support of pregnancy, and perhaps even parturition, are in part influenced by the expression of 5α-reductase type 1, and the products of its enzymatic activity. For any particular species, the significance of 5α-reductase in terms of a role in supporting pregnancy would depend crucially on the bioactivity of DHP at the PR. The existence of isoforms of the nuclear PR (Wei et al. 1988) is an equally important issue for consideration. Specifically, the results of human and rodent studies suggest that withdrawal might be associated with changes in the predominance of active and antagonistic PR isoforms, PR-A and PR-B (the “isoform switch hypothesis”; ISH), in addition to changes in cytokine and/or co-activator expression in myometrium and cervix (Mendelson 2009; Wagner et al. 2012; Fang et al. 2002; Merlino et al. 2007). Evidence for the existence of these PR isoforms has been obtained in cattle in early pregnancy (Slonina et al. 2012), but their possible involvement in functional progesterone withdrawal and the parturition cascade in ruminants or other domestic species remains unclear.

In addition to changes in PR isoforms, there are potentially other physiological effects of DHP or progesterone mediated through alternative transduction pathways. These ‘other pathways’ are often invoked to explain the rapid, non-genomic or non-translational effects of steroids, and indeed both classical and non-classical membrane-bound steroid receptors have been identified (Bernauer et al. 2001; Losel et al. 2003; Zhu et al. 2003; Karteris et al. 2006; Guerrero 2009). For example, membrane-coupled receptors responsive to progesterone (PGRMC1) have been found in myometrium of humans (Karteris et al. 2006) and other species (Slonina et al. 2012), and we have recently found expression of membrane estrogen and progesterone receptors in sheep endometrium and chorion during early pregnancy (days 14 through 30; Reynolds et al. 2012a; Reynolds et al. 2012b)). How physiologically important these and other non-traditional pathways of progestin action may be is unknown. Attempts to demonstrate direct bioactivity of DHP on both human and equine myometrial contractility in vitro (Lofgren & Backstrom 1994; Lofgren et al. 1992; Perusquia & Jasso-Kamel 2001; Ousey et al. 2000; Mesiano 2004) have been equivocal. Progesterone itself was unable to influence equine myometrial contractility in vitro (Ousey et al. 2000), though it has been shown to be inhibitory in similar studies with rabbit (CSAPO 1956; CSAPO & TAKEDA 1965) and human myometrium (Ruddock et al. 2008). The inhibition of human myometrial contractility by progesterone took hours to develop, and could not be blocked with a PR antagonist (Ruddock et al. 2008). Thus the effects of progesterone itself on myometrial contractility are neither immediate, as membrane receptor activation might be expected to be, nor are they likely mediated by classic nuclear PR and subsequent gene activation.

Even if DHP lacks direct effects on myometrial contractility, it may be a substrate for conversion to other bioactive pregnanes, which could occur in specific tissues and explain bioactivity in vivo that is not PR-mediated. Specifically, the 3α-reduced metabolite of DHP, allopregnanolone, is a potent gamma-aminobutyric acid (GABA) type A receptor agonist and “neurosteroid” that potentiates GABA itself when present at even low concentrations (Reddy 2003). Allopregnanolone has been shown to reduce human myometrial contractility within minutes of addition to muscle strips in vitro (Perusquia & Jasso-Kamel 2001). Similar relaxant effects have been reported for rabbit (Majewska & Vaupel 1991) and rat (Putnam et al. 1991) myometrium. Moreover, the circulating concentrations of allopregnanolone, and several
related pregnanolone isomers that increase during pregnancy (Parizek et al. 2005), decrease around the time of birth in women (Hill et al. 2001). Some of the effects of these neurosteroids may be central, mediated in part through oxytocin release (Leng & Russell 1999; Brussaard et al. 2000); less is known concerning tissue concentrations derived by local synthesis from DHP. Thus, whether or not DHP is a physiologically significant agonist of the PR in species other than horses, metabolism of DHP to allopregnanolone may mediate a physiological role in pregnancy and parturition. In this regard, we recently have shown that mRNA expression of one of the enzymes able to convert DHP to allopregnanolone, AKR1C3, can be regulated sheep fetal hypothalamus during late pregnancy (day 130, approximately 0.9 of gestation) by maternal nutrient intake (elevated in restricted- vs. control-intake ewes) as well as maternal treatment with melatonin (decreased in melatonin-treated vs. control ewes; LP Reynolds, AJ Conley, JS Caton, KA Vonnahme, & CO Lemley, unpublished observations). Levels of AKR1C3 mRNA also were present in sheep maternal (caruncular) and fetal (cotyledonary) placental tissues at parturition, but were 15-fold greater in maternal vs. fetal placenta (LP Reynolds et al., unpublished observations).

However it is that parturition is initiated, it must be timed so that the development of the fetus and its physiological preparation for birth is adequate to ensure the greatest likelihood of extra-uterine survival of the neonate. This requires dialogue between fetus and dam which, in the absence of neural connections, is necessarily hormonal in nature. The fetus either signals readiness for birth to the uterus, or the placenta signals impending delivery to the fetus. If successful, that dialogue culminates in maturation of the fetal adrenal cortex and the secretion of cortisol which is not only an element of the parturition process that is shared across all species studied, but is a prerequisite for neonatal survival. How fetal adrenal maturation and cortisol secretion is regulated remains one of the great mysteries of the physiology of birth (Liggins & Thorburn 1994).

Even though the gland is capable of responding to ACTH stimulation, fetal adrenal cortex is poorly organized and functionally quiescent for most of pregnancy (Conley & Assis Neto 2008). As term approaches however, the hypothalamic-pituitary-adrenal axis (HPAA) becomes activated (Wintour et al. 1975; Glickman & Challis 1980), though this is less dramatic in pigs (Silver & Fowden 1989; Conley et al. 1994) and difficult to demonstrate in the fetal foal (Silver & Fowden 1994). In addition, although fetal adrenal activation is required for fetal maturation and neonatal survival, it is not an initiator of the parturition cascade in all species. Pituitary aplasia prolongs pregnancy and prevents timely parturition in cattle (Stormont et al. 1956; Kendrick et al. 1957) and ewes (Liggins & Kennedy 1968; Liggins et al. 1967; Liggins & Thorburn 1994), but not reliably in primates (Liggins & Thorburn 1994; Novy et al. 1977; Mueller-Heubach et al. 1972). Therefore, there is clearly a spectrum across mammalian species with respect to the degree to which the fetal HPAA serves as the trigger for initiating birth even though fetal adrenal cortisol secretion is important for neonatal survival in all species known.

Little is understood about how activation of the fetal adrenal axis is regulated, but estrogens, prostaglandins and even neurosteroids have been implicated in providing both positive and negative stimuli (Conley & Assis Neto 2008). As noted above, the fetal adrenal is capable of responding to ACTH throughout gestation, and cortisol secretion by the fetal adrenal provides feedback on the hypothalamus in human (Goto et al. 2006) and ovine (Unno et al. 1998a) pregnancy. The sustained increase in fetal ACTH and cortisol that occurs over the last week or two of gestation in sheep (Brooks et al. 1989) and cattle (Comline et al. 1974) suggests a change in sensitivity to feedback. Results of studies in pigs indicate that placental tissue, if left in situ and still viable after fetal demise, can prevent a single remaining, viable fetus from initiating its own birth (Stryker & Dziuk 1975). Thorburn et al. postulated that the placenta somehow
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Steroidogenesis and the initiation of parturition influences the sensitivity of the fetal hypothalamus to negative feedback as fetal cortisol increases at term (Thorburn et al. 1991), and hormonal signals from the placenta have also been shown to activate the fetal HPAA. Specifically, estradiol (Wood 2005) and prostaglandin E2 (Challis et al. 1976; Fowden et al. 1987) secretion has been shown to increase in the ovine fetus in late gestation, both stimulate ACTH release (Young et al. 1996a; Young et al. 1996b) and cortisol secretion (Louis et al. 1976) and can induce parturition (Young et al. 1996a; Wood 1999; Wood & Saoud 1997) in this species. Conversely, inhibition of prostaglandin synthesis delays activation of the fetal HPAA (Unno et al. 1998b; McKeown et al. 2000; Gersting et al. 2008). The effect is likely above the level of the pituitary because hypothalamic disconnection severely mutes the response (Young et al. 1996b). If estradiol and/or prostaglandin E2 are key elements acting in the hypothalamus to activate the HPAA, as the results of some studies suggest, the cellular targets and mechanisms have yet to be identified.

The involvement of neurosteroids in modulating the physical activity of the ovine fetus in utero has been convincingly demonstrated (Nicol et al. 2001) and the potential for regulating the HPAA has been suggested (Conley & Assis Neto 2008; Brunton et al. 2014). Some of the most compelling data are those of Yawno et al. (Yawno et al. 2009), who showed that inhibition of 5α-reductase activity by carotid infusion of the enzyme inhibitor finasteride induced an immediate increase in fetal cortisol concentration. Finasteride has been shown to decrease neurosteroid concentrations in the brain of rats (Mukai et al. 2008), and GABA<sub>¿</sub> receptor agonists have been shown to modulate their HPAA (Mikkelsen et al. 2008). Additionally, the increase in fetal cortisol induced by finasteride in the fetal lambs was almost completely blocked by simultaneous infusion of alfaxalone, which is an 11keto-derivative of the neurosteroid allopregnanolone (Yawno et al. 2009). Allopregnanolone is among the most potent of the GABA<sub>¿</sub> receptor modulators (Belelli & Lambert 2005). It is present in the fetal brain and suppresses fetal arousal (Crossley et al. 1997). Stress increases allopregnanolone concentrations in fetal brain, and concentrations fall at birth (Hirst et al. 2006), suggesting that placental pregnanes may well provide a source of substrate for synthesis locally in the fetal brain. Although concentrations of allopregnanolone are not dependent on the fetal adrenal (Nguyen et al. 2004), it is unclear whether or not fetal adrenal activation or neonatal survival are influenced by circulating or local CNS levels of allopregnanolone. The extent to which placental pregnane synthesis contributes to circulating neurosteroid concentrations or feeds local synthesis in the fetal hypothalamus is unknown. The state of adrenal activation in mice deficient in 5α-reductase has not been reported (Mahendroo & Russell 1999), but allopregnanolone has been shown to suppress the adrenal axis in late pregnant rats (Brunton & Russell 2011). A mechanistic convergence between or among estradiol, prostaglandin E2 and neurosteroid synthesis in the fetal hypothalamus has not been reported but all are likely modulators of adrenal activation in the fetus.

The involvement of 5α-reductase in the parturition cascade may extend beyond the fetal HPAA and have relevance purely in the context of progesterone metabolism and physiological withdrawal. Whether or not fetal adrenal activation is required to initiate birth, and clearly in some species it is not (Novy et al. 1977), maternal progesterone (or progestin) withdrawal is likely a universal requirement across mammals. 5α-reduction of progesterone to inactive metabolites (unable to bind or activate PR of that species) may be critically important in some species. Evidence reviewed previously argues that increased steroidogenic enzyme expression and placental estrogen synthesis induced by cortisol cannot impact placental progesterone production significantly because of the large difference in total circulating mass of progestins compared with estrogens (Conley & Assis Neto 2008). If progesterone withdrawal does not involve decreased synthesis, it must involve increased metabolism to inactive products or changes in receptor or effector pathways (Merlino et al. 2007; Karteris et al. 2006; Mendelson...


The effectiveness of PR receptor antagonists in disrupting pregnancy is variable dependent on stage of pregnancy and species, but can either induce abortion or facilitate the abortifacient effects of prostaglandins in domestic animals (Hoffmann & Schuler 2000; Shenavai et al. 2012), primates (Baird 1993; Spitz et al. 1996) and rodents (Elger et al. 2000). This argues for a primary role for PR activation in pregnancy. As indicated above, the PR has evolved (Baker & Uh 2012) to exhibit differential binding and perhaps activation by various pregnanes (Jewgenow & Meyer 1998; Kontula et al. 1975). Immuno-assays cannot distinguish the various pregnanes, even if their bioactivity was defined in a particular species. In other words, even if measurable, the physiological significance of changes in pregnane concentrations relies on characterization of the bioactivities of these metabolites, and differences in biopotencies among species are to be expected. Progesterone withdrawal, even at a systemic level, cannot be evaluated until these steroids can be measured and their bioactivities are known. If 5α-reduction of progesterone generates inactive metabolites, the induction of this enzyme in uterine or placental tissues could well initiate progesterone, or progestin, withdrawal.

The significance of progestin withdrawal may extend beyond species in which placental progesterone secretion is sufficient to maintain pregnancy even to those that are luteal-dependent. Systemic progesterone concentrations decrease dramatically after day 28 of pregnancy in pigs (Robertson & King 1974), due in part to uterine uptake and presumably metabolism (Magness et al. 1986). This decline continues throughout gestation, and fetal adrenal activation occurs in late gestation (Randall & Tsang 1986) before there is any evidence of luteolysis. Uterine prostaglandin release is not evident until the day of farrowing (Silver et al. 1979). The increase in prostaglandins, which induces luteolysis, is preceded by a decline in progesterone associated with increasing fetal cortisol and an increase in maternal estrogens (Ford et al. 1998). In goats, the array of progesterone metabolites is large and increases as gestation proceeds (Linzell & Heap 1968; Sheldrick et al. 1981). Even if prostaglandin release completes luteolysis in pre-partum does, it is preceded by a significant decline in progesterone, consistent with the onset of parturition (Ford et al. 1999; Ford et al. 1998; Ford et al. 1995). Thus, increased pregnane metabolism may provoke progestin withdrawal in goats.

Pregnane metabolism also has been investigated in sheep. Anderson et al (Anderson et al. 1975) demonstrated increased placental 17α,20α-dihydroxyprog-4-ene-3-one in late gestation, which increased in maternal plasma after dexamethasone administration (Flint et al. 1975). Related 3α,20α- and 17α,20α-dihydroxylated metabolites have been identified in late gestation bovine fetuses (Janowski 1994). In contrast, others have reported that 5α-reduction was the major route of progesterone metabolism during pregnancy in ewes (Tsang & Hackett 1979). The major phenotype of mice lacking 5α-reductase type 1 is a defect in parturition (Mahendroo et al. 1996). If species differ in the source of progesterone or other progestins that maintains pregnancy, the array of metabolites is likely to be broad. However, if 5α-reduction of progesterone in the fetal hypothalamus suppresses fetal adrenal activation, but promotes progestin withdrawal in the placenta, the effects of its inhibition or ablation will differ depending on its relative importance in certain species. Inhibition of 5α-reductase would promote premature parturition if the fetal HPAA, freed of suppression by neurosteroids, activates and can initiate birth, consistent with Yawno et al (Yawno et al. 2009). Conversely, 5α-reductase inhibition would delay parturition if 5α-reduction of progesterone is an important route of progestin withdrawal in the placenta and uterus (Fig. 2). Finasteride, a potent 5α-reductase inhibitor, induced parturition in mares even though systemic DHP concentrations were not decreased and progesterone was increased by treatment (Ousey et al. 2001). Conversely, we reported that finasteride treatment prolonged gestation in spotted hyenas (Conley & Assis Neto 2008), but no effect was seen on gestation length in rats (Mann 2006). However, we have shown recently that fetal hypothalamic 5α-reductase type 1 mRNA can be up-regulated by maternal melatonin treatment (Reynolds et al. 2013) and that both 5α-reductase
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Steroidogenesis and the initiation of parturition type 1 and 2 are expressed at 3- to 4-fold greater levels in term placenta of sheep (LP Reynolds, AJ Conley, PP Borowicz & JS Caton, unpublished observations). The induction of 5α-reductase type 1 expression in sheep placenta by melatonin is particularly intriguing, because it suggests that the gene can be regulated, and in women and non-human primates (Jolly 1972) as well as in mares (Rossdale & Short 1967), labor most often occurs at night. Even though this is not the case in sheep (Lindahl 1964), there is still a nocturnal increase in melatonin in pregnant ewes and their fetuses (Yellon & Longo 1987).

Summary and conclusions

As a final note, neurosteroid synthesis is dependent on the continued metabolism of DHP to metabolites by 3α-reduction, whereas oxidative metabolism of progesterone or DHP via 20α- or 20β-hydroxylation is presumed to generate inactive metabolites. All of these reactions can be catalyzed by aldo-keto reductases (particularly those of the 1C family), which can also catalyze the synthesis of prostaglandins (Penning 1999). The aldo-keto reductases represent a large family of isozymes (Hyndman et al. 2003; Penning et al. 1997), and although they may be identified presumptively (and annotated) by transcript sequence, their activities are unlikely.
to be totally conserved across species. Nevertheless, they have been implicated in the timing of parturition (Byrns 2011). More importantly perhaps, they also represent a potential metabolic cross-road of steroid and prostanoid conversion (Fig. 3), and may play an especially pivotal role therefore in both progesterone withdrawal and prostanoid-stimulated uterine contractility. An increase in AKR1C expression might accelerate the metabolism of progesterone and the synthesis of prostaglandin, for instance. Alternatively, if substrate availability of one pathway were to increase markedly, the increased competition for available enzyme might decrease metabolism through the other pathway. The possible involvement of AKR1C isoforms in both prostanoid and pregnane metabolism is of both great interest and potential significance. In addition, as noted, we have detected substantial levels of AKR1C3 mRNA during late pregnancy in sheep fetal hypothalamic and placental tissues that can be regulated (Reynolds et al. 2013). What role(s) these play in fetal preparation for birth and initiation of parturition, however, remains to be defined.

Fig. 3. Diagrammatic representation of prostanoid (modified in part from Dozier et al. 2008) and progesterone pathways of metabolism, potentially linked by aldo-ketoreductase 1C (AKR1C1/2/3) isoforms shown, together with cyclo-oxygenase (COX) and 5α-reductase (SRD5A1/2) isoforms and prostaglandin dehydrogenase (PGDH), shown in blue. The AKR1C3 isoform potentially acts as a central regulator of myometrial contractility and fetal HPAA activity via its ability to convert DHP to its 3α-reduced metabolite, allopregnanolone, which is a potent gamma-aminobutyric acid (GABA) type A receptor agonist and thus hyperpolarizes excitable tissues and neurons. AKR1C3 also is able to convert PGH2 directly to PGF2α, and thus one alternative name for the enzyme is PGF synthase. See text for further explanation. Abbreviations: AKR = aldo-ketoreductase; COX = cyclo-oxygenase; DHP = dihydroprogesterone; GABA = gamma-aminobutyric acid; HPAA = hypothalamic-pituitary-adrenal axis; PG = prostaglandin; PGDH = prostaglandin dehydrogenase; PGFM = PGF metabolite; PGRMC = progesterone receptor membrane component; PR = progesterone receptor; SRD5A = steroid 5α-reductase.

Acknowledgments

The authors wish to acknowledge their colleagues (Drs. Joel Caton, Anna Grazul-Bilska, Dale Redmer and Kimberly Vonnahme, North Dakota State University, and Dr. Caleb Lemley, Mississippi State University) who helped to generate, and allowed the use of, unpublished data
referred to in this manuscript, and others who provided feedback and insight on drafts during preparation (Dr. Ian Bird, University of Wisconsin-Madison).

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