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Review

Review of the reproductive endocrinology of the pregnant and parturient mare

A.J. Conley*

Department of Population Health & Reproduction, School of Veterinary Medicine, University of California, Davis, California, USA

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A B S T R A C T

Analytical advancements, especially methods using gas or liquid chromatography tandem mass spectrometry, have allowed more specific and reliable measurement of multiple steroid hormones in the plasma of mares throughout gestation and the periparturient period. Data such as these will form the central focus of this review. The comprehensive analyses possible with liquid chromatography tandem mass spectrometry illuminate the key physiological and developmental transitions that make equine gestation unique. Weeks 6 to 20 encompass endometrial cup formation and equine chorionic gonadotropic secretion that stimulates primary corpora lutea and induces formation of secondary luteal structures. The period is defined by increased progesterone, 17OH-progesterone, and androstenedione secretion, providing substrate feeding the rise in estrone sulfate that can be used as an aid in the diagnosis of pregnancy. The 5α-reduced metabolite of progesterone, dihydroprogesterone (DHP), parallels progesterone secretion during this period at less than half the concentration. After week 12, progesterone declines, but DHP concentrations continue to increase, exceeding progesterone by week 16, thereby defining the luteo-placental shift in pregnane synthesis from ovarian to primarily placental thereafter. The growth of fetal gonads begins around week 14 and is defined by increasing dehydroepiandrosterone, among other androgens, which fuels placental estrogen secretion, functioning as a true fetoplacental unit. Metabolites of DHP (including allopregnanolone) dominate in late gestation, some exceeding DHP by 10-fold near term. However, all major pregnanes decrease from 3 days before foaling, when fetal cortisol is reportedly rising. Though unique, equine pregnancy and parturition share many features in common with those seen in human pregnancy and birth.

1. Establishing pregnancy

The physiology of pregnancy in mares is unique and, once pregnancy is established, many of the unusual phenomena that follow, and together make it so different from other species, are reflected in endocrine profiles in systemic blood which will constitute the major focus of this review. However, it is true that even before the endocrinology of pregnancy diverges from that of cyclicity in mares, pregnancy-specific phenomena are evident. Some of these are shared by other species or taxa, but the combination of those found in equine pregnancies is truly unique. As first reported by van Niekerk [1], the preferential retention of unfertilized oocytes in the oviducts [2] marks the first physiological response of the maternal tract to the presence of a conceptus. This is perhaps the earliest maternal response to pregnancy recognizable among mammals and has been described otherwise only among bats [3,4]. This event likely reflects the capacity of even early equine embryos to synthesize prostanoids [5,6] with effects on oviductal smooth muscle [7,8], although it is a response that is undetectable in systemic blood. Embryonic transuterine migration (protected within a capsule [9] until

* Corresponding author. Tel.: 530-752-2128; fax: 530-752-4278.
E-mail address: ajconley@ucdavis.edu

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Day 21 [10], itself an unusual structure [11]) is extensive between Days 11 and 16 [12]. Again, migration is probably driven in large part by embryonic prostanoids [13], and is essential to prevent luteolysis [14], and, thus, bring about the maternal recognition of pregnancy in horses. Progesterone concentrations are not significantly different between early pregnant and cyclic mares [15–17], or between conceptive and nonconceptive cycles [18], before luteolysis in nonpregnant mares. Embryos are equally capable of substantial estrogen synthesis [19–22], although what contribution (if any) this makes to either embryo migration or maternal recognition of pregnancy remains uncertain [23,24]. In any case, embryonic migration [14] is important in distributing, throughout the uterine lumen, a signal that suppresses prostaglandin secretion [25] by delaying endometrial expression of prostaglandin synthases [26], facilitating extension of luteal function sufficient for pregnancy to be established. Because none of these early events is clearly distinguishable by way of signals detectable in systemic blood before luteolysis is initiated [27,28], they will not be considered further here.

2. Progestogenic support of pregnancy

It has long been accepted that progesterone is the hormone that alone is required to establish and maintain pregnancy. Progesterone is a pregnane meaning that, like cortisol, it has a 21-carbon steroidal structure. It is alone among steroids in having its own receptor; all other receptors are named for their corresponding steroid class, estrogen, androgen, gluco- or mineralo-corticoid. Because progesterone activates the so-called progesterone receptor (PR), it is a progestin, meaning it is a bioactive pregnane by virtue of being progestogenic. To be clear, not all pregnanes are progestins; though both are pregnanes (21-carbon steroidal structure), pregnenolone (the "universal precursor") is devoid of bioactivity and cortisol has little or no progestogenic activity despite being a potent corticoid receptor agonist. Similarly, not all progestins are pregnanes; most synthetic progestins have acyl additions and other substituent modifications to a 21- or 19-carbon backbone or completely different molecular structure. Pregnanes can also have significant biological effects not involving classical nuclear steroid receptor activation (effects at plasma membrane receptors, see below, allopregnanolone and GABA receptor activation), although these types of responses have been best characterized in the context of studies on reproductive function conducted to date. Ovarian progesterone secretion is required to support pregnancy in mares for the first 50 to 70 days of gestation [29]; luteolysis must be prevented in order for progesterone secretion to be sustained and pregnancy to be established [27,28,30,31]. However, over half a century ago, Short [32] observed that progesterone was undetectable in the second half of gestation in mares, and pregnancy was supported presumptively by other pregnanes with progestogenic bioactivity, by alternate progestins as defined previously. Others confirmed this observation using assays with much greater sensitivity [33–35]. As progesterone concentrations decline in mid-gestation, the concentrations of a variety of 5α-reduced metabolites of progesterone increase to extremely high levels in late gestation [36]. The actual progestogenic bioactivity of these 5α-reduced pregnanes has been the subject of speculation for decades; whether or not they are true, progestins has remained in question. Support for presuming their bioactivity was obtained by comparing their ability to compete with progesterone for binding to extracts of equine tissues expressing the classic nuclear PR, endometrium [37,38], and mammary gland [38]. Of those investigated, dihydroprogesterone (DHP), formed from progesterone by direct 5α-reduction, was shown to compete with progesterone fairly equally [37,38], but attempts to demonstrate bioactivity failed [39]. Because antagonists bind to receptors with high affinity to block bioactivation [40], demonstrating actual progestogenic bioactivity of DHP was crucial. Scholtz et al. [41] recently demonstrated that DHP administered daily was sufficiently progestogenic to maintain pregnancy in mares until gestation day (GD) 27 after luteal regression was induced by prostaglandin on GD 14. These data firmly established the physiological significance of DHP as a progestin in the horse.

The studies by Scholtz et al. [41] used liquid chromatography tandem mass spectrometry to distinguish progesterone and DHP (few immunoassays can), together with an *in vitro* bioassay using the cloned equine progesterone receptor (ePR) to compare their relative progestogenic biopotencies directly for the first time. Consistent with the results of competitive binding studies using extracts of equine tissues, biopotencies were found to be comparable in reporter assays using the cloned ePR [41]. Dihydroprogesterone is presumed to play a similar role in the zebra [42], as well as elephants and even the related rock hyrax [43–46]. Furthermore, Scholtz et al. established

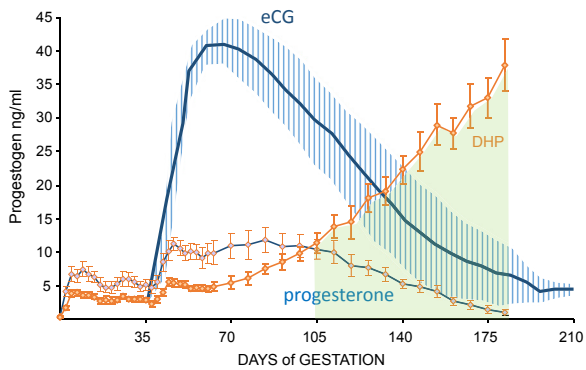

Fig. 1. Systemic plasma concentrations of progesterone and 5α-dihydroprogesterone (DHP) in relation to spike in equine chorionic gonadotropin (eCG) secretion in maternal plasma during the first half of equine gestation. Note, the appearance of eCG (≈Day 37 of pregnancy) coincides with revival of progesterone secretion by the primary corpus luteum (CL), with a parallel increase in DHP. Progestin concentrations are supplemented subsequently by secretion from induced secondary luteal structures. The increasing contribution of the placenta-to-progestin secretion is marked by increasing DHP as luteal secretion wanes and progesterone concentrations decline. The point at which DHP concentrations exceed those of progesterone (≈Day 105–110 of pregnancy) marks the luteo-placental shift (shaded). The transition from progesterone to DHP dominance might well serve as a rational end point for deciding when it is safe to discontinue supplemental progestin therapy. Data were adapted from Scholtz et al. [41] and Hoffmann et al. [47].