AN ABSTRACT OF THE THESIS OF

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In order to study normal and abnormal progestin biosynthesis, 15 newborn lighthorse and pony foals (325 to 358 d gestation) were either given synthetic ACTH (.2 IU/kg BW, i.v., n = 3), ACTH together with a 3ß-hydroxysteroid dehydrogenase blocker (trilostane) (10 mg/kg BW, p.o., n = 7), or no treatment (n = 5) at 4 h of age. Plasma samples were taken from birth through 96 h of age and assayed for cortisol by radioimmunoassay, and for pregnenolone, 5-pregnene-3ß,20ß-diol (P5ßß), 5-pregnene-3ß,20α-diol (P5ßα), progesterone, 5α-pregnane-3ß,20ß-diol (5α-DHP-ßß), 5α-pregnane-3ß,20α-diol (5α-DHP-ßα), 3ß-hydroxy-5α-pregnan-20-one (3ß-OH-5α-DHP) and 20ß-hydroxy-4-pregnen-3-one (20ß-P4) by gas chromatography/mass spectrometry. Preliminary comparisons demonstrated no significant differences in cortisol or progestin concentrations between normal foals given ACTH and ACTH/trilostane treatments and these groups were combined for statistical purposes. In normal foals cortisol increased from birth (99.54 ± 7.44 ng/ml, mean ± SE) to

maximum (124.18 ± 28.89 ng/ml) within 1 h of birth and rapidly declined, increasing again if treated with ACTH (129.04 ± 14.23 vs 24.11 ± 2.72 ng/ml at 6 h, for ACTH and normals, respectively) ($P < .05$); cortisol was below 11 ng/ml by 48 h. Pregnenolone, 5α-DHP-ßß, 5α-DHP-ßα, and 3ß-OH-5α-DHP were maximal at birth in normal foals (2.32 ± .39, 1.16 ± .68, .75 ± .40, .41 ± .07 μg/ml, respectively) with P5ßα maximum at 2 h (.51 ± .09 μg/ml) and P5ßß maximum at 4 h (6.30 ± 1.39 μg/ml). All progestins gradually decreased through 24 h in normal foals; ACTH treatment had no effect on progestin concentrations ($P > .05$). Three dysmature foals (336 to 352 d gestation) had lower ($P < .001$) cortisol at birth (25.17 ± 8.66 ng/ml) and failed to respond to ACTH ($P < .05$); cortisol later increased and remained elevated through 48 h (31.98 ± 10.87 ng/ml) ($P < .05$). In dysmature foals P5, P5ßß and P5ßα concentrations were lower ($P > .05$) at birth than normal foals, but steadily increased to a maximum of 2 to 8 times normal values at 24 and 48 h. Concentrations of 5α-DHP-ßß were not different at birth ($P > .05$), but 12 times higher in dysmature vs normal foals (3.31 ± .16 vs .21 ± .17 μg/ml at 24 h. 5α-Pregnane-3ß,20α-diol and 3ß-OH-5α-DHP gradually declined but tended to remain higher than normal in dysmature animals only. 20ß-Hydroxy-4-pregnen-3-one was elevated in dysmature animals only. Progesterone was not detected in either normal or

dysmature foals. Data suggest that in normal foals cortisol production declines and progestins clear rapidly from circulation within the first 48 h. Dysmature foals demonstrate lower cortisol and progestin values at birth and elevated progestins, especially 20ß-hydroxylated compounds, at 24 to 48 h. These aberrations in steroid metabolism may be due to enzymatic deficiencies, adrenal disfunction, impaired hepatic and(or) renal clearance or possibly organ immaturity. Further study is needed to determine specific causes before clinical treatments can be developed and evaluated for treatment of dysmaturity.

Cortisol, Pregnene and Pregnane Profiles in Normal and Dysmature Newborn Pony and Lighthorse Foals

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INTRODUCTION

The events that precede the initiation of parturition involve close synchronization between fetal and maternal systems. It is believed that the normal fetus, upon reaching a level of maturity sufficient to ensure survival in an extra-uterine environment, is responsible for the initiation of the birth process.

Central in fetal involvement is the maturation of the fetal pituitary-adrenal axis. In sheep (Liggins, 1968), cattle (Comline et al., 1974), pigs (First and Bose, 1979), rabbits (Kendall and Liggins, 1972) and horses (Nathanielsz et al., 1975) corticosteroids, in response to adrenocorticotropic hormone (ACTH) from the anterior pituitary (Martin, 1985), begin to increase just prior to parturition. Cortisol (11ß-17,21-trihydroxy-4-pregnene-3,20-dione) has been implicated in completion of lung maturation, surfactant production, hepatic glycogen storage, gut maturation and activation of enzyme systems in the fetus (Jost, 1966; Liggins, 1976; Silver, 1990).

In contrast, other adrenal steroids such as 3ß-hydroxy-5-pregnen-20-one (pregnenolone; P5) and pregnenolone metabolites are often elevated in dysmature

foals, those foals considered to be of adequate gestational age but appearing physiologically premature (Houghton et al., 1991). 17α-Hydroxypregnenolone and 17α-hydroxyprogesterone has also been shown to be in higher concentrations in premature human infants when compared to healthy, full term infants (Murphy et al., 1983; Lee et al., 1989).

Production of steroids such as cortisol and 17α-hydroxyprogesterone from pregnenolone is controlled by several enzymes including 17-hydroxylase, 21-hydroxylase, 11-hydroxylase and the 3ß-hydroxysteroid dehydrogenase isomerase complex (3ß-HSD). Whereas adrenal 17-, 21- and 11-hydroxylase appear to be active as early as 135 d in the rhesus monkey (term = 165 d) (Sholl, 1982), there was a 7-fold increase in 3ß-HSD activity at 155 to 165 d of gestation versus 135 d (Sholl, 1983). Blockage of 3ß-HSD by an inhibitor will cause an increase in plasma pregnenolone levels with a corresponding decrease in corticosteroids in several species (Potts et al., 1978; Lambert et al., 1984).

Little is known about the actual mechanisms involved in the induction of the birth process, although recent studies indicate that lesions of the paraventricular nucleus in fetal lambs results in prolonged gestation (McDonald et al., 1991). Exogenous administration of

glucocorticoids will cause both premature organ maturation and premature parturition in several species, including the horse (Alm et al., 1975; Silver, 1990), possibly mimicking the prepartum rise seen prior to birth (Liggins, 1976). Both premature human infants and foals tend to demonstrate aberrations in steroid levels, such as low glucocorticoids and elevated progestins (Murphy et al., 1983; Silver et al., 1984; Lee et al., 1989; Houghton et al., 1991). However, little is know about steroid biosynthesis and enzymatic mechanisms in both the normal and premature neonate, particularly at birth and in the early neonatal period.

Neonatal death due to prematurity often results in both financial and emotional hardship. In humans, premature births due to diabetes, twinning or idiopathic causes may require prolonged medical support due to inadequate lung development (respiratory distress syndrome) or other complications. Foals also may require expensive medical treatment if premature, or may not survive at all if assistance is not immediately available. Although helpful, gestational age is not a reliable indicator of fetal maturity in the horse (Rossdale and Silver, 1982) as individual variation is large. Therefore, physiological and behavioral criteria are needed for correct identification and treatment of

prematurity. In addition, although some parameters have been set in clinical evaluation of the premature neonate, little information has been available on characteristic hormone profiles. Gas chromatographic-mass spectrometric (GC/MS) techniques have been recently employed to identify and quantify plasma progestins of the equine neonate (Holtan et al., 1991; Houghton et al., 1991).

REVIEW OF LITERATURE

The initiation of parturition and survival of the fetus involve close synchronization between fetal and maternal systems. Maternal prepartum events in the horse include increasing development of mammary glands (Ousey et al., 1984), relaxation of the cervix (Hillman, 1975), and a decrease in plasma 4-pregnene-3,20-dione (progesterone; P4) (Pashen, 1984).

It is believed that the fetus is ultimately responsible for many of the events initiating birth. Glucocorticoids, such as cortisol in the horse (James et al., 1970), begin to rapidly increase just prior to parturition and in the mammalian fetus has been shown to be essential in organ maturation. In addition, glucocorticoids are thought to be responsible for the shifting of progesterone:estrogen ratios at the placental level and initiation of the hormonal events leading to parturition itself (Anderson et al., 1975).

Normal foals exhibit several behavioral and physiological characteristics indicative of a successful crossover into extra-uterine life. Physically, foals are able to independently assume the functions of respiration, locomotion and ingestion required of an adult. Soon after birth, there is rapid development of motor and sensory

functions (Leadon et al., 1986; McCall, 1991) Within 2 h, most foals are able to stand, move and nurse without assistance (Rossdale, 1967).

In contrast, premature or dysmature individuals are poorly prepared for the adjustment to independent life. Premature foals (< 300 days gestation) exhibit no coordinated righting reflex, the suck reflex is poor or absent, and respiratory insufficiency may be present (Leadon et al., 1986). Premature human infants often exhibit respiratory distress due to a lack of sufficient alveolar surfactant production to maintain adequate respiratory competency (Avery and Mead, 1959).

Premature human neonates and foals tend to exhibit abnormalities in endocrine profiles as well. Abnormally low plasma cortisol has been seen in premature foals (Rossdale et al., 1982). Conversely, elevated pregnenolone and pregnenolone metabolite (5-pregnene-3,20-diol) levels have been demonstrated in equines (Houghton et al., 1991), and elevated 17α-hydroxyprogesterone, 17α-hydroxypregnenolone and dehydroepiandrosterone sulfate levels in premature human infants at 2 to 5 d of age (Lee et al., 1989). These unusual steroid levels may suggest adrenal cortical dysfunction or immaturity, perhaps due to enzyme deficiencies or reduced steroid metabolism. Premature infants with respiratory distress syndrome

(indicative of lung immaturity), have smaller adrenal glands in comparison to normal infants of equal weight, which may be a result of reduced number of cortical cells (Naeye et al., 1971).

Hypothalamic-Pituitary-Adrenal Axis

Hypothalamus and pituitary. The neuroendocrine pathways of the fetus that are involved in the final maturation process and ultimately birth are thought to begin at the hypothalamic level. Lesions of the fetal hypothalamus (paraventricular nucleus) resulted in prolonged gestation in sheep, possibly due to a resultant decrease in corticotropin-releasing factor (CFR) (Gluckman et al., 1991; McDonald et al., 1991). As in the adult, CRF is released into the hypothalamo-hypophysial portal system and is carried to the pars distalis of the pituitary gland, where it stimulates the production and release of ACTH (Martin, 1985). ACTH stimulates adrenal cortical cells to produce glucocorticoids such as corticosterone and cortisol from cholesterol (Stone and Hechter, 1954; Martin, 1985).

The adrenal gland. In both the adult and the fetus, the adrenal gland constitutes one of the major endocrine glands of the body. Glucocorticoids are biosynthesized

from cholesterol in the adrenal cortex and are important for glucose metabolism, immune functions, acid-base balance and many other essential physiological functions (Martin, 1985). In most mammalian species, the adult adrenal cortex is divided into three concentric zones that surround the adrenal medulla (Black, 1988). The outermost zone, the zona glomerulosa, lies adjacent to the connective tissue encapsulating the adrenal and produces aldosterone, a mineralocorticoid. The central zone, the zona fasiculata and the zone that immediately surrounds the adrenal medulla (zona reticularis) produce glucocorticoids and androgens (Black, 1988). Catecholamines (epinephrine and norepinephrine) are produced by modified sympathetic ganglion cells in the adrenal medulla.

In contrast, the fetal adrenal cortex is differentiated into a definitive (adult) cortex, and an inner fetal cortex. The fetal adrenal produces significant quantities of sulfated hormones, such as dehydroepiandrosterone-sulfate (DHAS). These sulfated hormones may serve special functions, such as protecting the mother from hyperadrenocorticism during pregnancy due to the additional source of steroids (the fetus). Sulfated steroids are more rapidly degraded because of elevated microsomal enzyme activities during pregnancy

(Martin, 1985). The fetal cortex, although making up the bulk of the adrenal cortex prior to parturition, degrades rapidly after birth (Black, 1988).

Unlike the adult cortex, the fetal cortex produces little cortisol until close to term, possibly due to low activity of key enzymes such as 3ß-HSD. Fetal adrenal 3ß-HSD activity is concentrated in the outer cortical zone, which is thought to differentiate into the adult cortex after birth (Sholl, 1983; Black, 1988). Microsomal fractions of human fetal adrenal cells cultured with steroid substrates show greatest activity utilizing 17α-hydroxypregnenolone as a substrate (Hirato et al., 1982). In addition several steroids including estriol, estradiol, androstenediol and estradiol-17-sulfate were shown to inhibit enzymatic conversion of 17α-hydroxypregenolone to 17α-hydroxyprogesterone by 50% or more (Hirato et al., 1982), which may suggest a regulatory effect of certain steroid products. Although 3ß-HSD activity in the fetal adrenal increases with gestational age, other adrenal enzymes such as 17-hydroxylase, 21-hydroxylase and 11-hydroxylase appear to remain constant throughout pregnancy (Sholl, 1982). This suggests activation of 3ß-HSD may be important for initiation of the pre-parturient cortisol surge.

Steroidogenesis

Nomenclature. Cholesterol and all steroids share a parent nucleus of three six-membered rings coupled with a five-membered ring. The carbons and rings of this perhydrocyclopentanophenanthrene structure are sequentially numbered from left to right in accordance with IUPAC nomenclature (Anon, 1969) (Figure 1a).

When the ring-structure of a steroid is viewed as a projection on to a plane of paper, an atom or group attached to a ring is termed α (alpha) if it lies below the plane of the paper, or ß (beta) if it is oriented above the plane of the paper (Figure 1b). Stereoisomers, isomers whose constituent atoms are bonded identically, but their atomic arrangement in space differs, are important since structural differences such as spatial configuration may result in radically differing biochemical functions due to changes in bonding sites. Saturated (i.e. with no double bonds present between ring carbons) or unsaturated compounds (double bonds are present) may also result in radically different hormonal effects, as well as differences in position of double bonds, and ketone or hydroxyl moieties. Saturated steroids (identified by an '-ane' ending) are thought to be reduced products and less biologically active.

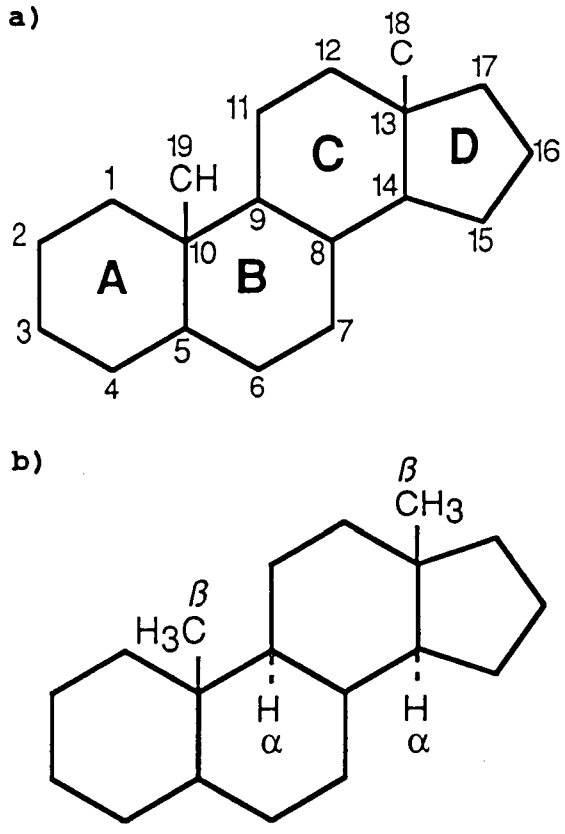

Figure 1a and 1b. Steroid hormone structure and nomenclature

Biosynthesis. Corticosteroids such as glucocorticoids (cortisol, corticosterone), sex steroids (progesterone, estrogen and testosterone), and mineralocorticoids (aldosterone) are biosynthesized from cholesterol in response to ACTH stimulation. In the adult, the adrenal gland and gonads are the major source of steroid synthesis; the placenta also serves as a temporary source of steroids (progestins and estrogens) for the fetus. In mammals, cholesterol is supplied through the diet or biosynthesized in the liver from acetyl coenzyme A. Side-chain cleavage of cholesterol by cholesterol ester hydrolase and cholesterol desmolase results in the oxidation of carbons 20 and 22 and the formation of isocaproyl aldehyde and another C21 steroid in the form of pregnenolone. The oxidation and cleavage of the cholesterol side-chain is accelerated by the presence of ACTH and is a rate-limiting step for steroid biosynthesis (Martin, 1985).

Pregnenolone is converted to progesterone by the shifting of a double bond and removal of a hydrogen and is facilitated by 3ß-HSD (Martin, 1985). In addition, several metabolites such as 5-pregnene-3ß,20ß-diol (P5ßß) and 5-pregnene-3ß,20α-diol (P5ßα) may also be produced. In several species, 3ß-HSD activity in the fetal adrenal is low until close to the end of gestation, with 17α-

hydroxypregnenolone the preferred substrate (Pepe et al., 1977; Hirato et al., 1982; Sholl, 1983). 17α-Hydroxyprogesterone is then formed from 17α-hydroxypregnenolone through hydroxylation. Other steroids, such as dehydroepiandrosterone, progesterone and estriol have been shown to inhibit 3ß-HSD activity (Hirato et al., 1982). In the placenta, 3ß-HSD utilizes pregnenolone as a substrate for progesterone production, and substantial amounts of fetal glucocorticoids, such as cortisol, are also produced at the placental level from further metabolism of pregnenolone and progesterone.

Progesterone is one of the major intermediates in the synthesis of all major steroids from the gonads and adrenals. 5α-pregnanes (progesterone metabolites) have been found in higher concentrations in the umbilical artery and vein when contrasted with maternal vessels in the horse (Holtan et al., 1975). Further work by Moss et al. (1979) suggested that 3ß-hydroxy-5α-pregnan-20-one (3ß-OH-5α-DHP) (another progesterone metabolite) is primarily formed by the fetus. Holtan et al. (1991) identified primarily P5, 3ß-OH-5α-DHP, P5ßß and 5α-pregnane-3ß,20ß-diol (5α-DHP-ßß) in the fetal artery, while 5α-DHP and progesterone were found in the fetal vein. Uterine venous and arterial steroids tend to mirror those detected in maternal circulation but at higher

concentrations (Holtan et al., 1991). This suggests fetal pregnenolone is converted to progesterone and(or) 5α-DHP by the placenta, with further 3ß- and 20ß- hydroxylation by the fetus. Low conversion of progesterone to 5α-DHP was found by Hamon et al. (1991) in placental incubations, indicating that 5α-DHP may result from the metabolism of a steroid other than progesterone.

Progesterone and 17α-hydroxyprogesterone are converted to 11-deoxycorticosterone (DOC) and 11-deoxycortisol, respectively, by the addition of an alcoholic group through the action of 21-hydroxylase. DOC undergoes additional hydroxylation at the 11 position to yield corticosterone, the major adrenal steroid seen in rats, mice, birds, reptiles and amphibians (Hadley, 1984). Further metabolism by 18-hydroxylase results in the formation of aldosterone, the major mammalian mineralocorticoid.

11-Deoxycortisol is metabolized by 11-hydroxylase to form cortisol, the major adrenal steroid in most mammals, including humans and equines (James et al., 1970; Hadley, 1984).

A brief overview of steroid biosynthesis is provided in Figure 2. Systematic and trivial names of selected steroids are listed in Table 1.

Significance of Progestins

In mammals, progesterone is essential for the maintenance of pregnancy. In those animals where the corpus luteum (CL) is the primary source of progesterone, loss of the CL will result in abortion. In the mare and ewe, studies have indicated that placental progesterone is adequate to maintain pregnancy without luteal sources of progestagens by Day 50 of gestation (Thornburn et al., 1977; Holtan et al., 1979). 3ß-Hydroxy-5ß-pregnan-20-one, 5ß-pregnane-3,20-dione, 3α-hydroxy-5α-pregnene-20-one and progesterone all inhibit spontaneous uterine contractions *in vitro* in rat uterine tissue, and may be one of the mechanisms responsible for the inhibition of uterine contractility during implantation and pregnancy (Putnam et al., 1991). This appears to be through modulation of the inhibitory transmitter γ-aminobutyric acid (GABA) complex at a site distinctly different from that used by barbiturates (Harrison et al., 1987; Peters et al., 1988; Turner et al., 1989).

Blockage of 3ß-HSD in pregnant ewes by infusion of epostane or trilostane (Winthrop-Breon Laboratories, New York, NY), competitive inhibitors of 3ß-HSD, results in significant reduction of plasma progesterone and can prematurely induce parturition (Taylor et al., 1982;

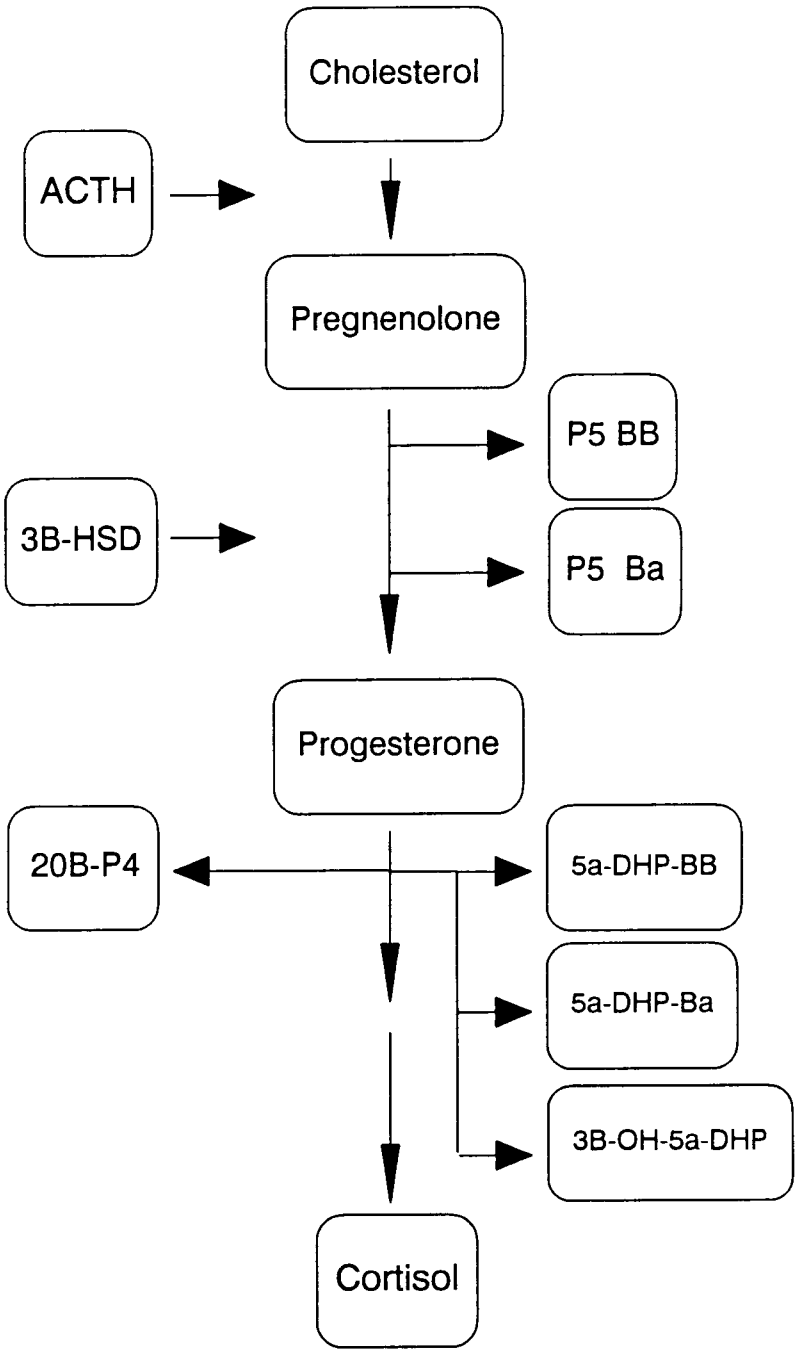

Figure 2. Steroid biosynthesis. See Table 1 for full systematic names.

Table 1. Systematic and trivial names of selected steroids.

Systematic Name	Trivial Name (Abbreviation)
5-cholesten-3ß-ol	Cholesterol
3ß-hydroxy-5-pregnen-20-one	Pregnenolone (P5)
5-pregnene-3ß,20ß-diol	Pregnene-diols (P5ßß)
5-pregnene-3ß,20α-diol	Pregnene-diols (P5ßα)
4-pregnene-3,20-dione	Progesterone (P4)
5α-pregnane-3ß,20ß-diol	Pregnane-diols (5α-DHP-ßß)
5α-pregnane-3ß,20α-diol	Pregnane-diols (5α-DHP-ßα)
3ß-hydroxy-5α-pregnan-3-one	(3ß-OH-5α-DHP)
11ß,17,21-trihydroxy-4-pregnene-3,20-dione	Cortisol

Silver, 1988). However, Fowden and Silver (1987) could not prematurely induce parturition in the mare by the use of epostane, even though a significant decrease in plasma progesterone in both the uterine artery and vein was observed. This may be due to species differences in the steroidogenic pathways between the ewe and the mare, even though the placenta is the primary source of progesterone in both species in late pregnancy.

Role of Glucocorticoids

In all mammals, glucocorticoids such as cortisol are closely linked with the onset of parturition. In sheep, cattle, goats, pigs, horses, and rabbits a dramatic rise in fetal glucocorticoid levels occurs a few days before birth (Liggins, 1976; Silver, 1990). A similar, although less abrupt, increase is seen in humans (Smith and Shearman, 1974). Glucocorticoids act on many tissues in the body, with target tissues all demonstrating the presence of glucocorticoid receptors. Glucocorticoid receptors in the hypothalamus and pituitary of fetal sheep are greatest on approximately Day 100 of gestation (term = 147 d), but pituitary receptors increase again near term when endogenous cortisol is elevated (Yang et al., 1990).

Glucocorticoids induce enzyme activity, which in the adult assists in maintaining homeostasis. However, in the fetus, glucocorticoids also are involved in organ development, activation of enzymatic systems, and tissue differentiation and are closely linked with the maturation of the lung and gut, as well as with hepatic glycogen storage in the fetus (Silver, 1990).

Lung Maturation. Studies have shown that the fetal lung is especially suited as a target organ for glucocorticoids as it contains both nuclear and cytoplasmic receptors in higher numbers than either the fetal liver or kidney (Ballard and Ballard, 1974). Additionally, studies in rabbits, rats and guinea pigs have demonstrated that corticosterone in the lung is readily converted to cortisol. Functional maturation of the lung is judged by the presence or absence of alveolar surfactant, a surface active lipoprotein that reduces the surface tension in the lung and prevents alveolar collapse (Avery and Mead, 1959). Respiratory distress syndrome (RDS; hyaline membrane disease) is a developmental disease seen in premature human infants and animals where the ability to breathe is severely compromised due to a marked absence of lung surfactant.

Until recently, the action of glucocorticoids was thought to be a direct effect upon the alveolar type II

cells of the lung. Alveolar II cells are responsible for the production of lung surfactant. Recent work has shown that cortisol is involved in indirect stimulation of choline-phosphate cytidyleltransferase activity through fibroblast-pneumonocyte factor (Post et al., 1986). Choline-phosphate cytidyleltransferase is the enzyme primarily involved in the production of alveolar surfactant in the lung.

In the mouse fetus, lung surfactant activity is a late developmental feature (Buckingham et al., 1962). Rabbit fetuses treated in utero with corticosteroids demonstrate increased pulmonary maturation (Kotas et al., 1971). Premature human infants demonstrate decreased lung compliance and reduced surfactant levels. In addition, infants with RDS tend to exhibit lower adrenal weights than normal infants (Naeye et al., 1971). Premature fetal lambs given ACTH to stimulate glucocorticoid production, show an increase in lung maturation as judged by the presence of alveolar surfactant. Prepartum maternal administration of exogenous glucocorticoids in women anticipating premature delivery has been shown to reduce the effects of RDS (Liggins and Howie, 1972). In addition, premature infants with severe RDS given dexamethasone (a synthetic glucocorticoid) demonstrated reduced lung damage and were able to be removed from

pulmonary support earlier than untreated infants (Yeh et al., 1990).

Small Intestine. In utero, the fetus obtains most of its energy from maternal glucose. Upon birth, stored fetal glycogen is only sufficient to supply fetal needs for several hours. Therefore, adequate maturation of the gastrointestinal system is essential for survival.

Fetal lambs show significant maturation of the small intestine just prior to parturition, at the time of the pre-partum cortisol surge. Villus height and base width, mucosal thickness, muscle thickness, and submucosal thickness appear to be, at least in part, controlled by corticosteroids (Trahair et al., 1987). Lamb fetuses that were bilaterally adrenalectomized at approximately 124 d gestation (term = 147 d) showed significant decreases in mucosal thickness, villus height, and villus base width, when compared to controls. Increases in muscle and submucosal thickness were also apparent (Trahair et al., 1987). Exogenous cortisol given to the fetus well before the pre-partum cortisol surge (97 d gestation) induced precocious changes in villus enterocyte kinetics similar to that found much later in gestation (Trahair, et al., 1987). Neonates of many species obtain passive immunity through absorption of maternal antibodies found in colostrum. Gut closure, which refers to the loss of

ability of the neonatal small intestine to allow macromolecules, such as antibodies, to pass through into circulation may be affected by glucocorticoids. In the rabbit and guinea-pig glucocorticoids increase at or just before the time period associated with gut closure (Malinowska et al., 1972).

Activation of invertase, an important gastrointestinal enzyme, occurs just prior to parturition in several species. Newborn rats injected with cortisol at 9 d of age induced detectable invertase activity at a much earlier stage in development (Doell and Kretchmer, 1964). Although not proven to be directly related to cortisol, rises in glucose-6-phosphatase and other pancreatic and intestinal digestive enzymes occur just prior to birth near the time of the cortisol rise.

Liver Glycogen Storage. As with many other organs, rapid changes occur in the liver, specifically with glycogen storage, just prior to birth. In rats, rabbits and horses the amount of glycogen present rises suddenly just prior to parturition (Jost, 1966; Fowden et al., 1991). This may be due to either an increased sensitivity of the liver to glucocorticoids, the rapid increase in glucocorticoids with the liver already prepared to respond, or a combination of these two events. Exogenous corticosteroids given to decapitated 18.5 d rat fetuses

stimulate glycogen storage (Jost, 1966). Twelve times less glycogen was found in livers from 28 d rabbit fetuses that were decapitated 9 d before, when compared to controls (Jost, 1966).

Cortisol given to pregnant rats will cause a premature rise in glycogen stores in the fetus. Glycogen levels equal to that of a 19.5 d fetus can be induced in a 17.5 d fetus treated with exogenous cortisol. ACTH given to 17 d fetuses also provoked an increase in liver glycogen, suggesting that the increase in glycogen storage may be in response to increased adrenal secretion.

Enzymes involved directly with the synthesis of hepatic glycogen, such as phosphoglucomutase (PGM) and uridine diphosphoglucose-glycogen transglycosylas (UDPG-Tr), as well as those enzymes involved in degradation of glycogen (glucose-6-phosphase) show a sharp increase in late gestation in the rat (Jacquot and Kretchmer, 1964). In decapitated rat fetuses from adrenalectomized mothers UDPG-Tr and glucose-6-phosphate did not exhibit this characteristic increase (Jacquot and Kretchmer, 1964).

Prematurity and Dysmaturity

Early parturition, whether spontaneous or through induction, is often coupled with an increase in the

occurrence of premature neonates. It is therefore important to be able to identify those individuals whose physiological maturity is inadequate for survival of the early postnatal period and initiate appropriate supportive treatment.

Gestational Age. In animals in which gestation length varies only by hours (rodents, rabbits) or days (sheep, pigs) the final maturation processes of the fetus occur over a relatively short period of time. In other species with longer gestational lengths, such as the horse and man (11 and 9 m, respectively), the period of final maturation occurs over a much wider time period. Particularly in the horse, there is considerable variation in "normal" gestational ages (Laing and Leech, 1975). Due to this individual variation, it is difficult to judge prematurity by gestational age. A comparison of two Thoroughbred foals (Rossdale and Silver, 1982) with markedly differing gestational ages (281 and 338 d) demonstrated that even with spontaneous deliveries diversity is great in both physiological and behavioral aspects. In this particular study, many parameters indicated a greater level of physiological maturity in the younger foal.

Studies have shown that there may be some correlation between gestational age and level of maturity. Parturition in mares induced by administration of synthetic glucocorticoids, prostaglandin or prostaglandin in combination with oxytocin demonstrated that foals with gestational ages of less than 300 d have a poor chance of surviving the postnatal period and tend to exhibit behavioral signs indicative of immaturity (Alm et al., 1975; Leadon et al., 1986). However, due to the discrepancies between level of maturity and actual gestation age, it may be somewhat inaccurate to judge level of maturity in the neonate by gestational age alone.

Physiological Development and Behavior. Behavior patterns in the normal neonatal foal tend to be somewhat chronological and predictable. Extreme deviations from these patterns, or even subdued behavior, may be indicative of prematurity or dysmaturity.

Normal, fully mature foals will lift their head and neck and attempt to obtain sternal recumbency within seconds after being born (McCall, 1991). This "righting reflex" usually results in the rupture of the amnion and assists in allowing the foal to breathe freely without encumbrance of the amnionic membranes. The umbilical cord is often broken as the foal continues to struggle and makes creeping movements away from the dam. Often these

movements progress within the next 15 to 30 min into attempts to stand as the foal's hindlegs develop sufficient strength and movement to assist in lifting its body off of the ground (McCall, 1991). Foals predictably will stand within 40 to 60 min after birth, although considerable variation (15 to 165 min) is also normal (Rossdale, 1967). Foals will also make attempts at walking during this time and normally by 2 h postpartum are able to walk normally (McCall, 1991).

Foals born with gestational ages of less than 300 d tended to exhibit no coordinated righting reflexes (Leadon et al., 1986). In extreme instances, assistance may be required for the foal to maintain sternal recumbency. Time to first standing may be delayed (Leadon et al., 1986). Premature foals often require assistance to stand, and may have difficulty maintaining independent balance and walking.

Foals have pupillary light reflex and react to bright flashes of light within 10 min of birth (Rossdale, 1967). Within an hour after being born, normal foals are able to track visual and auditory stimuli. Often this stimuli will be large, moving objects (e.g., the dam) and foals have been shown to innately follow these objects. The suck reflex, which refers to the foal aggressively searching for and recognizing the mare's teats and udder,

is generally strong and most individuals will actively investigate the underside of the mare's belly, chest and flank areas. If unassisted, most foals will locate and nurse without assistance.

Foals with gestational ages of less than 300 d may display nystagmus when their eyes are open and often demonstrate no active interest in their surroundings (Leadon et al., 1986). Premature foals often show a weak, transient or absent suck reflex. In some instances the normal coordinated sucking movements of the lips and tongue have been replaced with champing of the jaw (Leadon et al., 1986). In one study, two foals had signs of facial paralysis (Leadon et al., 1986).

Endocrinology. Abnormally low cortisol values have been seen in the premature horse at birth (Rossdale et al., 1982). Conversely, these profiles tend to be elevated when compared to normal equine neonates several hours postpartum. There is little information on the cause and result of these changes in cortisol, particularly when related back to similar changes in steroids and their metabolites earlier on in steroidogenesis. However, administration of synthetic glucocorticoids to women where premature delivery was an immediate concern has been helpful in decreasing the incidence of complications in premature infants,

reaffirming the importance of corticosteroids in fetal maturation and development (Liggins and Howie, 1972). In addition, the abnormalities seen in dysmature or premature foals may be related to the availability of precursors for glucocorticoid synthesis.

In normal foals, preliminary data from this study has shown that both pregnenolone and P5ßß concentrations are high soon after parturition (greater than 3 µg/ml and 7 µg/ml, P5 and P5ßß respectively), and rapidly clear to undetectable levels within the first day of life (Houghton et al., 1991). The high levels of steroids such as pregnenolone may be due in part to placental sources and(or) fetal adrenal production, and may be involved with the pre-partum glucocorticoid surge.

Premature foals have exhibited high plasma concentrations of pregnenolone (> 10 µg/ml P5 and > 50 µg/ml P5ßß) although progesterone is still generally undetectable. Pregnenolone then appears to be more slowly cleared or metabolized as elevated levels remain detectable up to several days postnatally in horses (Houghton et al., 1991). This may be due to clearance impairment and(or) an enzymatic malfunction (3ß-HSD) that delays the metabolism of pregnenolone and its metabolites. Enzymatic abnormalities, such as 3ß-HSD inactivity or suppression, may explain the rise in cortisol in dysmature

or premature foals as precursors are initially blocked and only later are available for steroidogenesis.

In normal infants, plasma levels of progesterone and 17α-hydroxyprogesterone are elevated at birth and decline by the first week of life (Sippell et al., 1980). Conversely, premature human infants exhibit elevated 17α-hydroxypregnenolone and 17α-hydroxyprogesterone levels when compared to normal, full term infants at 2 to 5 d of age (Murphy et al., 1983; Lee et al., 1989). Although this does not appear to be consistent with blockage of steroid metabolism early in the steroidogenic pathway (e.g., 3ß-HSD), this may be in part a result of delayed sampling. Pregnenolone and pregnene-diol profiles in dysmature foals show depressed plasma concentrations at birth when compared to normal foals and a subsequent increase in progestin levels after 24 h of age (Houghton et al., 1991).

SUMMARY

Steroids are the result of a metabolic process that begins with the conversion of cholesterol to pregnenolone. Steroidogenesis is stimulated in the adrenal gland by ACTH and is catalyzed by several rate-limiting enzymatic steps, such as the conversion of pregnenolone to progesterone by 3ß-HSD. Steroids such as cortisol (a glucocorticoid synthesized primarily in the adrenal gland) are essential for adequate fetal development, and ultimately in the birth process itself through their involvement in organ maturation and enzyme activation. Normal fetal development results in an individual that is well prepared to adapt to an extrauterine existence with physiological maturity that mirrors the adult. However, dysmature and premature neonates are poorly prepared to exist independently of maternal support. This is demonstrated both in behavioral aberrations as well as abnormal endocrine profiles. Dysmature foals tend to have little response to adrenal stimulation. Both glucocorticoid and progestin profiles are unusually depressed at birth and resemble those seen in the pre-parturient fetus. Premature human infants tend toward elevated 17α-hydroxypregnenolone and 17α-hydroxyprogesterone levels at 2 to 5 d of age.

Considering both the financial and emotional costs of support of premature neonates, study of the mechanisms and pathways involved in steroidogenesis of the fetal-placental unit is of extreme value. Understanding these mechanisms may provide insight as to the cause of dysmaturity as well as the optimal supportive therapy for neonatal survival.

Due to the ease of induction of parturition in the mare, the availability of compounds to selectively block 3ß-HSD activity, and the specificity of GC/MS, the objective of this study was to attempt to observe the effects of blockage of 3ß-HSD with and without adrenal stimulation by exogenous ACTH on plasma cortisol and progestins in normal foals within the first 96 h of life. In addition, effort would be made to identify and(or) characterize previously unknown progestins and progestin metabolites. Due to the availability of both normal and dysmature foals, additional work was undertaken to 1) study the effects of ACTH stimulation on both normal and dysmature foals, and 2) to identify plasma progestins characteristic of normal and dysmature foals during the first 96 h of life.

MATERIALS AND METHODS

Parturition

Parturition was either spontaneous (n = 7) or induced (n = 8) with oxytocin (Burns Veterinary Supply, Oakland, CA) in fifteen lighthorse and pony mares when the mare was judged to be within 12 h of spontaneous delivery. Readiness for birth was determined by length of gestation, mammary development (including waxing of the teats and the presence of milk), increasing milk calcium, relaxation of the sacroiliac ligaments, and dilatation of the cervix (Hillman, 1975; Ousey et al., 1984; Cash et al., 1985). Amount of cervical dilatation was determined by vaginal palpation and was considered adequate when 2 fingers (4 cm) could be easily inserted into the os cervix. In addition, changes in cervical mucous were noted. A rectal examination was also performed prior to induction to ensure normal presentation of the foal. All mares had been immunized against tetanus, equine herpesvirus 1, encephalomyelitis and influenza 1 mo prior to the expected parturition date (Hillman, 1975), and were housed at either the Oregon State University Horse Center or the College of Veterinary Medicine in box stalls and fed a pelleted alfalfa-grain diet supplemented with grass hay twice daily. Water was provided free choice. Upon satisfying the criteria for induction, the mares were

administered 20 to 40 U of oxytocin, i.v., followed by additional 40 U if first-stage labor was not observed within 30 min of the initial dose.

Upon the onset of second stage labor (lateral recumbency, contractions and appearance of the fetal membranes), foal position was checked by vaginal palpation to ensure correct positioning of the fetus in the birth canal. The mare was assisted in labor only if dystocia was diagnosed upon vaginal palpation, otherwise parturition was allowed to proceed unassisted.

At birth all foals were observed for evidence of normal adaptive responses such as sternal recumbency, evidence of suck reflex, standing, and degree of extension of the fetlock joints. Respiratory and cardiovascular abnormalities were also noted. Foals were weighed within 1 h of birth, and umbilical stumps were treated with a dilute tincture of iodine solution at birth and at 12 h postparturition.

Foals were either given synthetic ACTH (Park-Davis, Morris Plains, NJ; 0.2 IU/kg BW, i.v., n = 3) (Eiler et al., 1980), ACTH with trilostane (Winthrop-Breon, New York, NY; 10 mg/kg BW, p.o., n = 7) or no treatment

Table 2. Gestational ages, birth weights and treatments of 15 newborn lighthorse and pony foals. All parturitions were either induced (I) or spontaneous (S).

Foal	Gestational age (d)	Birth Weight (kg)	Birth	Treatment/Comments
Mayday	355	Not taken	I	Control, normal
Jeana	339	Not taken	I	Control, normal
Bing	335	Not taken	S	Control, normal
Slopes	339	Not taken	S	Control, normal
P#46	342	17.3	I	ACTH[a] only, normal
P#56	341	16.4	I	ACTH[a] only, normal
P#48	342	15.5	S	ACTH[a] only, normal
P#35	325	23.6	I	ACTH[a]/trilostane[b], normal
P#60	355	19.5	S	ACTH[a]/trilostane[b], normal
Pokey	329	50.0	S	ACTH[a]/trilostane[b], normal
Gypsy	335	59.1	S	ACTH[a]/trilostane[b], normal
Irene	358	45.5	S	ACTH[a]/trilostane[b], normal
P#43	348	13.6	I	ACTH[a]/trilostane[b], dysmature[c]
P#44	352	19.1	I	ACTH[a]/trilostane[b], dysmature[d]
Amy	336	Not taken	I	Control, dysmature[c,e]

a ACTH given at a dose of .2 IU/kg BW, i.v. at 4 h.
b Trilostane (modrostane) given at a dose of 10 mg/kg BW, p.o. at 4 h.
c Hypoflexion of pasterns at birth; suppressed suck reflex.
d Heart murmur present at birth and throughout the study period. Hypoflexion of front pasterns only.
e Hypoflexion of pasterns at birth; unable to stand and nurse without assistance through 48 h. Hyaline membrane disease present.

n = 5) at 4 h of age (Table 2). Trilostane was given in order to selectively block 3ß-HSD activity, with and without adrenal stimulation by exogenous ACTH. Dosages were determined on the basis of previous studies with trilostane and with related compounds (Fowden and Silver, 1987; Silver 1988).

Blood samples were taken via jugular venipuncture at 0, 0.5, 1, 2, 4, 4.5, 5, 6, 8, 12, 16, 24, 48, 72, and 96 h of age and from the umbilical artery and vein at birth when possible. Heparinized samples were centrifuged at 1500 x g for 15 min and the plasma removed and stored at -20°C until assayed. Age was estimated for foals whose time of birth could not be accurately determined due to unobserved births.

Quantification of Steroids in Plasma

Cortisol. Plasma samples were assayed for cortisol by radioimmunoassay (RIA; Diagnostic Products Company, Los Angeles, CA). Samples were assayed in duplicate and a high and low cortisol control sample of equine plasma was also tested with each assay to determine inter- and intra-assay precision.

Progestins. Progestin standards were purchased from Sigma Chemical Co., Poole, Dorset, U.K. and Steraloids

LTD, Croydon, U.K. Methoxyamine hydrochloride, dimethylformamide (DMF), diethylamine hydrochloride, N-(tert-butyldimethylsilyl)-N-methyl trifluoroacetamide (MTBSTFA), deuteromethanol (CH_3OD), sodium deuteroxide (NaOD), deuterium oxide (D_2O) and sodium borohydride were also obtained from Sigma Chemical Co. C18 Sep-Pak cartridges were purchased from Waters Associates, Milford, MA, U.S.A. Reagent or alanar grade solvents were purchased from Rathburn Chemicals, Ltd., Walkerburn, Scotland or Mallinckrodt Chemicals, St. Louis, MO (USA).

Deuterium labelled steroids were prepared for use as qualitative and quantitative internal standards by methods outlined by Dehennin et al. (1980). Deuterium labelled P5 (D_4-P5) and 3ß-OH-5α-DHP (D_4-3ß) were prepared from P5 and 3ß-OH-5α-DHP by proton/deuterium exchange in alkaline medium (CH_3OD-NaOD). Sodium borohydride reduction of D_4-P5 and D_4-3ß yielded D_4-5-pregnen-3ß,20ß-Diol (D_4-P5ßß), and D_4-5α-pregnane-3ß,20ß-diol (D_4-ßß-Diol), respectively. Oxidation of D_4-3ß with chromium trioxide in glacial acetic acid yielded D_4-5α-pregnane-3,20-dione (D_4-5α-DHP). Deuterated products were recrystallized in CH_3OD-D_2O and checked for purity and completeness of deuteration by full-scan gas chromatography/mass spectrometry (GC/MS) with a resultant increase of 4 mass units greater that the parent steroid.

After addition of Deuterium labelled internal standards (D_4-IS) to plasma samples and plasma standards (1 to 5 ml), H_2O was added at an equal volume, mixed, and the sample was allowed to equilibrate for 30 min. Samples were applied to activated (CH_3OH, H_2O) C18 Sep-Pak cartridges and rinsed with H_2O and hexane (5 ml and 3 ml, respectively). Steroids were eluted with diethyl ether (3 ml) and the eluate dried under nitrogen at 40°C.

Plasma samples and standard extracts were derivatized with methoxyamine HCL (5%, w/v in pyradine; 50 µl; 30 min at 60°C) and the pyradine was removed under nitrogen. Diethylamine HCL (5% w/v, in dimethylformamide, 50 µl) and 25 µl MTBSTFA was added and the mixture incubated at 80°C for 45 min. Water (.5 ml) was added to the derivatized steroids and the result extracted twice with 3 ml of ether and dried under nitrogen at 40°C. Addition of methoxyamine HCL and MTBSTFA resulted in the production of methoxime (MO) and(or) tert-butyldimethylsilyl (TBDMS) derivatives of steroids with oxo (MO) or hydroxyl (TBDMS) groups for identification by GC/MS. Steroids with 17-hydroxylation were not analyzed as derivatization was not possible using TBDMS. The resulting residue was then dissolved in 30 µl of undecane for GC/MS analysis.

GC/MS Analysis. GC/MS analysis was accomplished using a Hewlett Packard 5890 Gas Chromatograph partnered

with a Hewlett Packard 5970 Series Mass Selective Detector (Hewlett Packard Co., Palo Alto, CA). Steroid separation was carried out using a fused silica capillary column (18 m x .25 mm i.d., SE-30 or OV-1, Econocap, Alltech Associates, Carnforth, Lanc., U.K.) maintained at 150°C for .5 min, heated at 20°C/min to 240°C, then at 10°C/min to 310°C, with a total run time of 20 min per injection. Samples and standards were injected by autosampler in the splitless mode. The mass selection detector (MSD) was operated in the full-scan mode for initial identification and the selected ion mode (SIM) for routine quantification of steroids and their corresponding internal standards as listed in Table 3.

Standard regression response lines were established for each steroid by fortification of 3 ml charcoal-extracted gelding plasma with increasing amounts of standard (from 10 to 800 ng/ml) plus a constant amount (250 ng/ml) of deuterium labelled internal standards. Due to higher expected values, P5 and P5ßß were added at .5 to 10 µg/ml and their respective D_4-IS at 2.5 µg/ml. Pregnenolone, P5ßß, 5α-DHP, 3ß-5P and ßß-Diol were calibrated against their respective D_4-IS, while those without corresponding D_4-IS were calibrated with the most closely related D_4-IS as follows: P4 by D_4-5α-DHP, 20α-5P by D_4-ßß-Diol and ßα-Diol by D_4-ßß-Diol.

Table 3. Progestin standards and assay performance of GC/MS.

Systematic name	Retention time (min)	Ion Monitored	Recovery[a] %	Assay CV%[b] Intra-	Inter-
5α-Pregnene-3,20-dione (5α-DHP)	11.30 11.12	343	102.4 ± 2.1	7.5	5.8
4-Pregnene-3,20-dione (P4)	11.30	372	89.5 ± 6.7	9.6	22.1
3ß-Hydroxy-5-pregnene-20-one (P5)	12.84	402	95.4 ± 1.5	5.1	---
3ß-Hydroxy-5α-pregnan-20-one (3ß-OH-5α-DHP)	12.98	404	96.5 ± .9	3.0	3.7
20α-Hydroxy-5α-pregnan-3-one (20α-5P)	13.83 13.99	404	92.4 ± 6.3	2.9	26.0
5-Pregnene-3ß,20ß-diol (P5ßß)	16.49	489	92.0 ± 1.9	6.7	---
5α-Pregnane-3ß,20ß-diol (5α-DHP-ßß)	16.67	491	105.4 ± 1.9	3.2	7.5
5α-Pregnane-3ß,20α-diol (5α-DHP-ßα)	17.08	491	97.7 ± 4.1	5.8	7.0

[a] Recovery of known concentrations of progestins added to charcoal-extracted gelding plasma.

[b] Intra- (n = 7) and inter- (n = 6) assay coefficents of variation.

Quantitation of the selected endogenous steroids was accomplished by integration of the peak areas for the selected ions and their corresponding internal standards using a Hewlett Packard Series 200 Computer with Ver. 3.1 MSD software coupled with user written MACRO programs. Least squares linear regression was calculated for each steroid standard and their regression equations used in a data base program to calculate concentration of each endogenous steroid.

Statistical Analysis

Data from all foals (n = 15) were used for cortisol analysis. Foals that had complete (0 through 96 h) sample sets (n = 8) were further analyzed for plasma progestin content. Statistical analysis of steroid levels was performed by conventional methods (means ± SE) and by repeated measures ANOVA for time, treatment, and time by treatment interactions. Where applicable, comparison of means by Student's paired t-test was also used. Due to the nature of the experimental design, a separate analysis was performed on the first 4 h of treatment (pre-ACTH and(or) trilostane) and the time periods (4 h through 96 h) after treatment. For statistical analysis, data from

all normal animals, regardless of treatment, are combined for times 0 (birth) through 4 h as no treatments were given until after 4 h.

RESULTS

Parturition and Maturity

All animals were considered near term by gestational age (320 to 360 d, n = 15) and all except one survived the neonatal period (Table 2). Parturition in eight of fifteen mares was induced by administration of exogenous oxytocin when mares were considered within 12 h of parturition using previously published criteria (Hillman, 1975). All other mares delivered spontaneously. Average ages of foals delivered by induction were not different ($P > .05$) when compared to spontaneously delivered foals (342.3 ± 9.6 vs 341.9 ± 10.8 d, respectively). There was also no significant difference in gestational age between normal and dysmature foals (341.3 ± 3.0 vs 345.3 ± 5.8 d, respectively).

Twelve foals exhibited normal behavioral and physiological responses at birth as measured by time to first standing, suck reflex, time to first nursing, response to external stimuli, respiration rate, heart rate and endocrine profiles.

Three dysmature foals (336, 348, and 352 d) tended to be less vigorous at birth and exhibited suppressed suck reflexes. Pony Foal #43 (PF#43) required assistance at

birth due to moderate dystocia; the lighthorse foal (Amy) was delivered following placenta previa. PF#43 and Amy demonstrated hypoflexion in all four pasterns; Amy was also unable to stand without assistance through the first 48 h of life. An abnormal heart sound was detected in pony foal #44 at birth, and remained audible upon auscultation throughout the study period. All dysmature foals survived the neonatal period except for Amy who died at 48 h of age and was diagnosed with hyaline membrane disease at necropsy. General behavior and response to stimuli in all dysmature foals agreed with previously published observations for premature or dysmature foals (Leadon et al., 1986).

Assay

Cortisol. Standards and sample assay variability (means ± SE, coefficients of variation and linear correlation) were determined by conventional statistical methods. Standard curves exhibited a linear response (r^2 > .996) over a range of 10 to 500 ng/ml. Mean inter-assay coefficients of variation were 3.2 and 5.5% (n = 5) for both high (150 ng/ml) and low (80 ng/ml) sample pools, respectively. Intra-assay coefficient of variation for the low sample pool (n = 12) was 5.4%.

Progestins. Retention times, percent recovery of known concentrations of progestins added to plasma and intra- and inter-assay coefficients of variation for steroid standards are listed in Table 3. All D_4-IS eluted slightly ahead of their corresponding non-labelled standards. 5α-Pregnanes and 20α-5P demonstrated doublet peaks due to the syn- and anti-isomers of the methyloxime at the C-3 position. Closely eluting peaks (e.g., P5 and 3ß-OH-5α-DHP) were differentiated by selected ion monitoring (Table 3) and identification confirmed by full-scan fragment patterns (Appendix B). Occasionally difficulty in quantification of 3ß-OH-5α-DHP and ßß-Diol occurred with the presence of extremely high levels of P5 and P5ßß, respectively, due to detector saturation. These cases were resolved by dilution and re-analysis of the samples.

Standard ratios exhibited a linear response (r^2 > .96) over a range of 2 to 800 ng/ml (P5 and P5ßß, .1 to 2.0 μg/ml). Depending upon volume and extraction efficiency (50 to 70%), overall assay sensitivity was at least .5 ng/ml. Extraction efficiency did not affect quantitation due to the use of D_4-IS. Recoveries (mean ± SE) of steroids added to charcoal-extracted gelding plasma are listed in Table 3. Intra- and inter-assay coefficients of variation for a pooled plasma sample from

late gestation fortified with a known amount of steroids (9.1 ng P4/ml to 101.1 ng 20α-5P/ml) were < 10% for all steroids (n = 7 and 6, respectively), except for P4 (22.1%) and 20α-5P (26.0%).

Steroids

Preliminary comparisons demonstrated no significant differences in cortisol or progestin concentrations between normal foals given ACTH and ACTH/trilostane treatments and these groups were combined for statistical purposes (Table 2). As there were also no differences observed between Dysmature/ACTH verses Dysmature/no ACTH these data were also combined for statistical analysis. Data for individual foals is listed in Appendix A.

Cortisol. Plasma cortisol concentrations increased from 99.54 ± 7.44 ng/ml at birth and were maximum (124.18 ± 28.89 ng/ml) by 1 h in normal foals and declined rapidly to baseline levels (≤ 11.0 ng/ml) by 48 h (Figure 3). In those foals treated with ACTH at 4 h (.2 IU/kg BW, i.v), cortisol again rapidly increased to a peak of 132.66 ± 10.14 ng/ml within 1 h of ACTH treatment ($P < .05$), and thereafter declined to baseline levels by 24 h (Figure 3). There was an effect of treatment ($P < .05$) as well as a

significant effect of time in ACTH treated foals when compared to normals from birth through 48 h.

In contrast, mean plasma cortisol concentrations were lower at birth ($P < .001$) in dysmature foals when compared to normal foals (25.17 ± 8.66 vs 99.54 ± 7.44 ng/ml, respectively) and remained suppressed ($P < .05$) through the pre-ACTH treatment period. Maximum concentration (43.02 ± 12.27 ng/ml) was reached at 6 h, five hours later than normals, after a slower more gradual increase (Figure 3). There was a significant effect of dysmaturity when compared to either normal or ACTH treated groups. There was also an effect of time ($P < .05$), although through the first 48 h concentrations tended to remain relatively constant between 30.00 and 40.00 ng/ml (Table 4). A minimum of 14.64 ± 6.69 ng/ml was observed at 96 h.

5-Pregnenes. Pregnenolone concentrations were maximum at birth in normal foals (2.32 ± .39 μg/ml), rapidly declined to less than 1.00 μg/ml by 12 h and were undetectable by GS/MS analysis at 96 h (Figure 4); ACTH treatment at 4 h had no effect.

Dysmature foals tended to exhibit lower ($P = .09$) plasma pregnenolone levels at birth (1.13 ± .39 μg/ml vs 2.32 ± .39 μg/ml), but overall were not different when compared to normal foals through 48 h ($P > .05$). As seen in normals, pregnenolone concentrations in dysmature foals

declined to approximately 1.20 μg/ml by 6 h, but in contrast were elevated ($P < .05$) at 48 h and tended to remain high through 96 h (Table 5). Maximum concentrations were seen at 72 h ($n = 2$), and were more than 20 times that seen in normal foals (2.68 ± 2.21 vs .03 ± .01 μg/ml, respectively) (Table 5).

P5ββ increased from 3.09 ± 1.02 μg/ml at birth to a maximum of 6.30 ± 1.39 μg/ml at 4 h in normal foals and gradually decreased to less than .30 μg/ml by 48 h (Figure 5). In contrast, plasma P5ββ concentrations were three times lower ($P > .05$) at birth in dysmature foals (.96 ± .16 μg/ml at 0 h) and peaked much later (16.22 ± 7.28 μg/ml at 48 h) ($P < .05$) (Figure 5). There was a significant difference between normal and dysmature groups when compared from birth through 48 h. Although the greatest differences were seen from 4 through 48 h, there were significant effects of dysmaturity seen at all times beginning at 2 h through at least 48 h (Table 6). P5ββ concentrations were 5, 11 and 55 times higher in dysmature foals when compared to normals at 12, 24 and 48 h of age, respectively (Table 6).

In normal foals, P5βα demonstrated a pattern similar to that seen with P5ββ, increasing from birth to a peak of .51 ± .09 μg/ml at 2 h. Concentrations then gradually declined to less than .04 μg/ml at 48 h and were

undetectable by 72 h by GC/MS (Figure 6). As with P5ßß, plasma P5ßα levels tended to be lower at birth (P = .06) in dysmature animals when compared to normals (.15 ± .02 vs .46 ± .10 μg/ml, respectively). At 5, 6, 12 and 24 h, P5ßα levels were consistently higher (P < .05) in dysmature foals, and still tended to be elevated (.75 ± .31 vs .03 ± .01 μg/ml, respectively) at 48 h (P > .05). An overall effect of both time and treatment (P < .05) was seen as plasma P5ßα concentrations in dysmature foals tended to be higher than in normal foals from .5 h and steadily increased over time. P5ßα levels were 8 to 10 times higher in dysmature vs normal animals at 24 (P < .05) through 48 h (P > .05) (Table 7).

4-Pregnenes and 5α-pregnanes. Little or no progesterone, 20ß-hydroxy-4-pregnen-3-one or 5α-DHP was detected in foals from either experimental group, however, progesterone metabolites (5α-pregnane-diols) were present in elevated concentrations, particularly in the dysmature animals (P < .05) (Tables 8 and 9).

5α-Pregnane-3ß,20ß-diol and 5α-DHP-ßα were maximum at birth in normal foals (1.16 ± .68 and .75 ± .40 μg/ml, respectively) and declined rapidly thereafter (Figure 7 and 8). In contrast, 5α-DHP-ßß and 5α-DHP-ßα peaked later in dysmature foals when compared to normal foals (12 h and 2 h, respectively) (Tables 8 and 9). There was no overall

effect of dysmaturity demonstrated from birth through 4 h for both 5α-DHP-ßß or 5α-DHP-ßα. Pregnane-diol levels were significantly elevated in dysmature foals when compared to normals after 4 h through the end of the study period ($P < .05$). 5α-Pregnan-3ß,20ß-diol was 12 times higher in dysmature vs normal foals (3.31 ± .16 vs .21 ± .17 μg/ml, respectively) at 24 h and 5α-DHP-ßα was also significantly elevated (.21 ± .09 vs .02 ± .01 μg/ml) at 24 h (Figures 7 and 8).

Plasma concentrations of 3ß-OH-5α-DHP were maximum (.41 ± .07 μg/ml) at birth in normal foals and rapidly declined within 48 h of age (Figure 9). There was no significant difference seen in dysmature foals when compared to normals at birth (.66 ± .28 vs .41 ± .07 μg/ml, respectively) (Table 10). Although overall concentrations remained slightly higher in dysmature foals, there was no difference between normal and dysmature foals throughout 48 h ($P > .05$).

20ß-Hydroxy-4-pregnen-3-one was occasionally detected in normal foals, but was not found before 1 h and was undetectable after 12 h by GC/MS. Highest concentrations were seen at 6 h (.07 ± .04 μg/ml). Overall concentrations were much higher in dysmature foals (Table

11) and remained detectable through at least 72 h. Highest concentrations of 20ß-hydroxy-4-pregnen-3-one were after 4 h (.38 ± .03 µg/ml at 24 h).

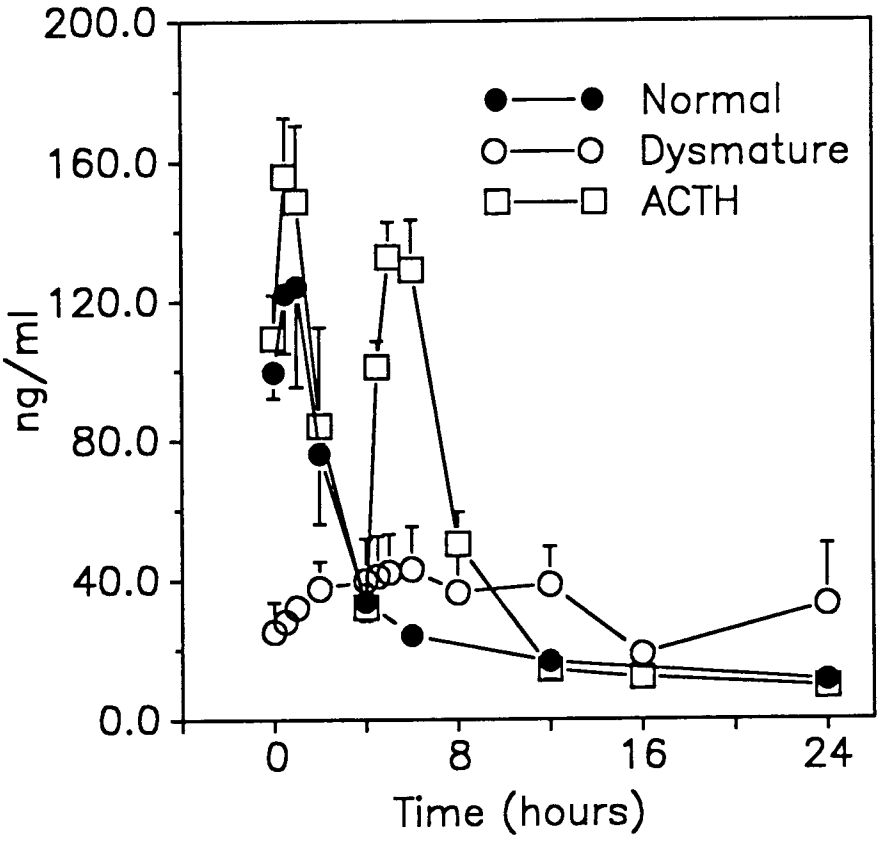

Figure 3. Plasma cortisol (mean ± SE) in normal (n = 4), ACTH treated (n = 5, .2 IU/kg BW, 4 h), and dysmature (n = 3) newborn lighthorse and pony foals.

Table 4. Plasma cortisol (ng/ml, mean ± SE) in normal (n = 4), ACTH-treated (n = 5, .2 IU/kg, BW, 4 h) and dysmature (n = 3) newborn lighthorse and pony foals from birth (time 0) through 96 h of age.

Time	Normals	ACTH	Dysmature
0	99.54 ± 7.44[a]	109.27 ± 12.55[a]	25.17 ± 8.66[b]
.5	122.21 ± 17.25[a]	155.87 ± 16.71[a]	28.09 ± 3.18[b]
1	124.18 ± 28.89[a]	148.72 ± 21.56[a]	32.15 ± 3.78[b]
2	76.07 ± 19.95[a]	84.05 ± 28.43[a]	37.66 ± 7.57[a]
4	33.71 ± 5.51[a]	32.22 ± 6.76[a]	39.72 ± 12.09[a]
4.5	---	100.99 ± 7.55[a]	41.03 ± 11.49[b]
5	---	132.66 ± 10.14[a]	42.20 ± 10.86[b]
6	24.11 ± 2.72[a]	129.04 ± 14.23[b]	43.02 ± 12.27[a]
8	---	50.27 ± 9.31[a]	36.61 ± 10.17[a]
12	16.57 ± 5.23[a]	14.27 ± 3.68[b]	38.73 ± 10.77[a]
16	---	12.05 ± 3.06	18.65 ± 3.32[¶]
24	11.14 ± 2.27[a]	9.36 ± 1.80[b]	33.13 ± 17.10[a]
48	10.17 ± 2.84[a]	8.91 ± 1.82[b]	31.98 ± 10.87[a]
72	8.16 ± 1.97[a]	8.50 ± 1.46[a]	15.60 ± 3.07[a]
96	9.91 ± 3.64[a]	9.35 ± 1.56[a]	14.64 ± 6.69[a]

[a,b] Values within a row followed by a different superscript are different ($P < .05$).

[¶] $n \leq 2$, not tested.

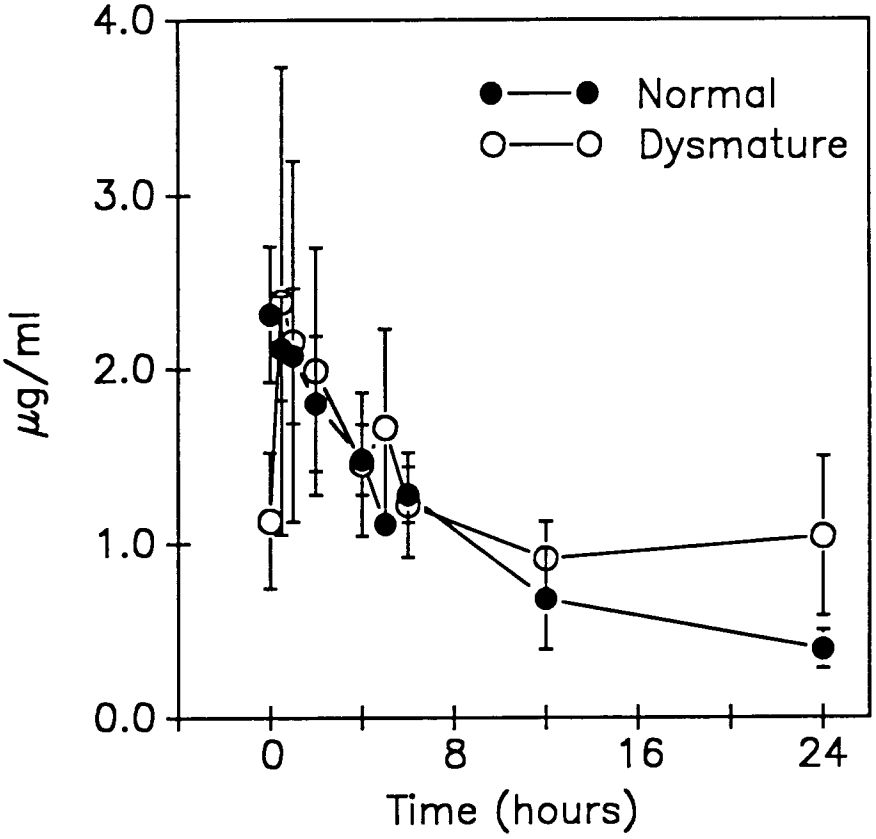

Figure 4. Plasma 3ß-hydroxy-5-pregnen-20-one (mean ± SE) in normal (n = 5) and dysmature (n = 3) newborn lighthorse and pony foals.

Table 5. Plasma 3ß-hydroxy-5-pregnen-20-one (µg/ml, mean ± SE) in normal (n = 5) and dysmature (n = 3) newborn lighthorse and pony foals from birth (time 0) through 96 h of age.

Time	Normals	Dysmature
0	2.32 ± .39*	1.13 ± .39*
.5	2.12 ± .30[a]	2.39 ± 1.34[a]
1	2.08 ± .39[a]	2.16 ± 1.04[a]
2	1.80 ± .39[a]	1.99 ± .71[a]
4	1.48 ± .20[a]	1.45 ± .41[a]
5	1.11 ± .09¶	1.66 ± .57¶
6	1.13 ± .16[a]	1.22 ± .30[a]
12	.68 ± .29[a]	.91 ± .21[a]
24	.39 ± .11[a]	1.04 ± .46[a]
48	.06 ± .01[a]	2.33 ± 1.19[b]
72	.03 ± .01	2.68 ± 2.21¶
96	BDL	1.09 ± 1.02¶

* P = .09
[a,b] Values within a row followed by a different superscript are different (P < .05).
¶ n ≤ 2, not tested.
BDL Below detection limit of assay.

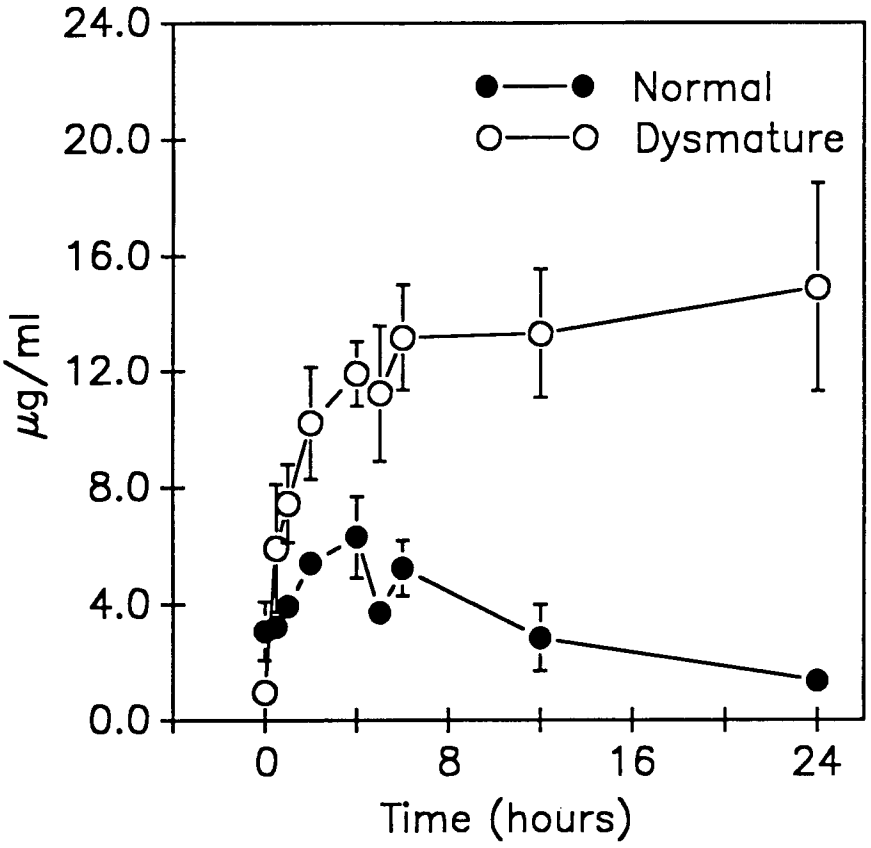

Figure 5. Plasma 5-pregnene-3ß,20ß-diol (mean ± SE) in normal (n = 5) and dysmature (n = 3) newborn lighthorse and pony foals.

Table 6. Plasma 5-pregnene-3ß,20ß-diol (µg/ml, mean ± SE) in normal (n = 5) and dysmature (n = 3) newborn lighthorse and pony foals from birth (time 0) through 96 h of age.

Time	Normals	Dysmature
0	3.09 ± 1.02[a]	.96 ± .16[a]
.5	3.23 ± .26[a]	5.92 ± 2.18[a]
1	3.94 ± .31[*]	7.45 ± 1.33[*]
2	5.41 ± .56[a]	10.21 ± 1.94[b]
4	6.30 ± 1.39[a]	11.92 ± 1.12[b]
5	3.73 ± .44[¶]	11.25 ± 2.22[¶]
6	5.23 ± .94[a]	13.17 ± 1.83[b]
12	2.85 ± 1.14[a]	13.31 ± 2.22[b]
24	1.37 ± .54[a]	14.91 ± 3.60[b]
48	.29 ± .06[a]	16.22 ± 7.28[b]
72	BDL	13.48 ± 11.45[¶]
96	BDL	4.53 ± 4.32[¶]

* P = .06
[a,b] Values within a row followed by a different superscript are different (P < .05).
¶ n ≤ 2, not tested.
BDL Below detection limit of assay.

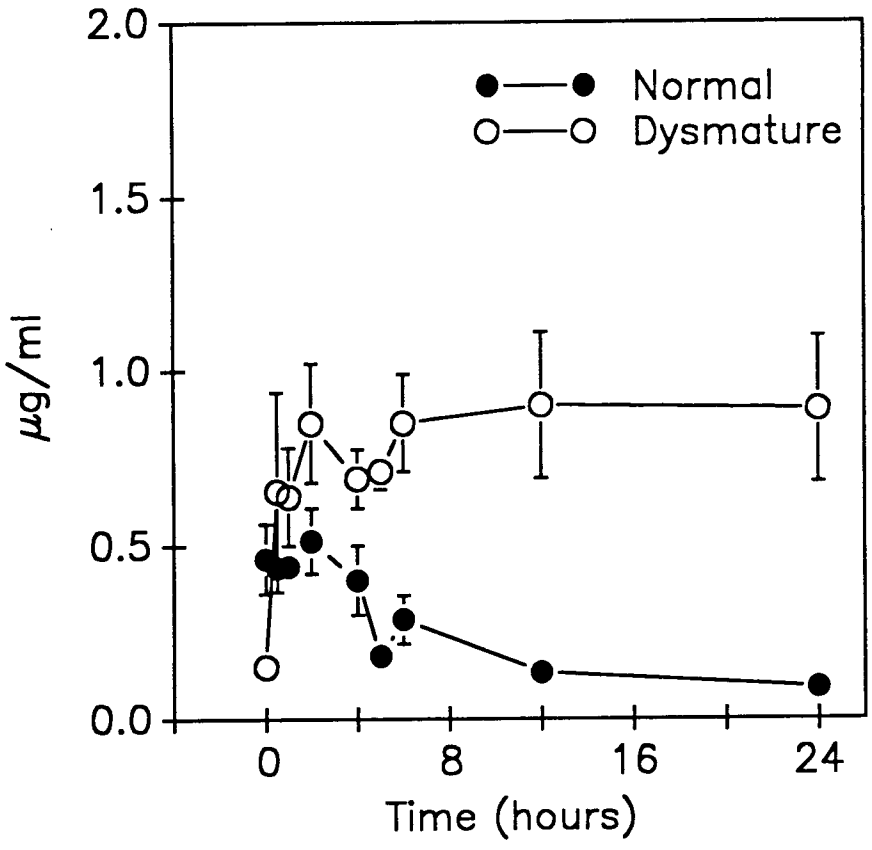

Figure 6. Plasma 5-pregnene-3ß,20α-diol (mean ± SE) in normal (n = 5) and dysmature (n = 3) newborn lighthorse and pony foals.

Table 7. Plasma 5-pregnene-3ß,20α-diol (μg/ml, mean ± SE) in normal (n = 5) and dysmature (n = 3) newborn lighthorse and pony foals from birth (time 0) through 96 h of age.

Time	Normals	Dysmature
0	.46 ± .10*	.15 ± .02*
.5	.44 ± .02[a]	.65 ± .29[a]
1	.44 ± .02[a]	.64 ± .14[a]
2	.51 ± .09[a]	.84 ± .17[a]
4	.40 ± .10[a]	.69 ± .08[a]
5	.18 ± .01[a]	.71 ± .05[b]
6	.29 ± .07[a]	.85 ± .14[b]
12	.13 ± .04[a]	.90 ± .21[b]
24	.09 ± .03[a]	.89 ± .21[b]
48	.03 ± .01[a]	.75 ± .31[a]
72	BDL	.48 ± .35¶
96	BDL	BDL

* P = .06
[a,b] Values within a row followed by a different superscript are different (P < .05).
¶ n ≤ 2, not tested.
BDL Below detection limit of assay.

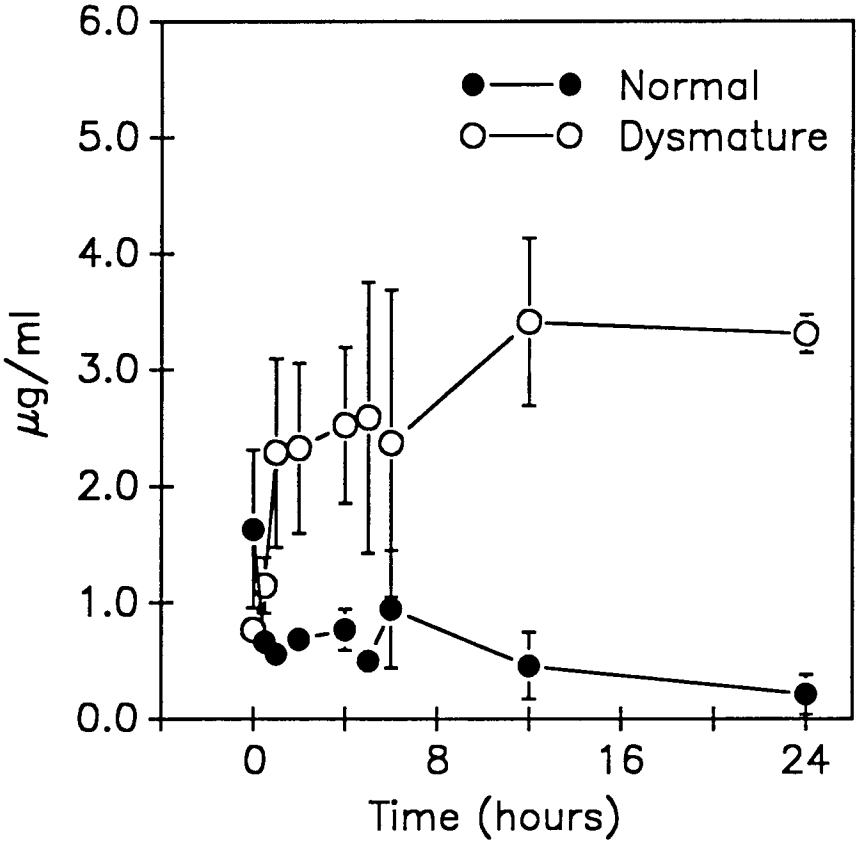

Figure 7. Plasma 5α-pregnane-3ß,20ß-diol (mean ± SE) in normal (n = 5) and dysmature (n = 3) newborn lighthorse and pony foals.

Table 8. Plasma 5α-pregnane-3ß,20ß-diol (μg/ml, mean ± SE) in normal (n = 5) and dysmature (n = 3) newborn lighthorse and pony foals from birth (time 0) through 96 h of age.

Time	Normals	Dysmature
0	1.16 ± .68[a]	.77 ± .09[a]
.5	.67 ± .14[a]	1.15 ± .24[a]
1	.56 ± .01[a]	2.29 ± .81[b]
2	.69 ± .13[a]	2.33 ± .73[b]
4	.77 ± .18[a]	2.53 ± .67[b]
5	.51 ± .01¶	2.59 ± 1.16¶
6	.95 ± .50[a]	2.37 ± 1.32[a]
12	.46 ± .29[a]	3.41 ± .72[a]
24	.21 ± .17[a]	3.31 ± .16[b]
48	.01 ± .01¶	2.37 ± .88¶
72	BDL	2.12 ± 1.93¶
96	BDL	BDL

[a,b] Values within a row followed by a different superscript are different ($P < .05$).
¶ $n \leq 2$, not tested.
BDL Below detection limit of assay.

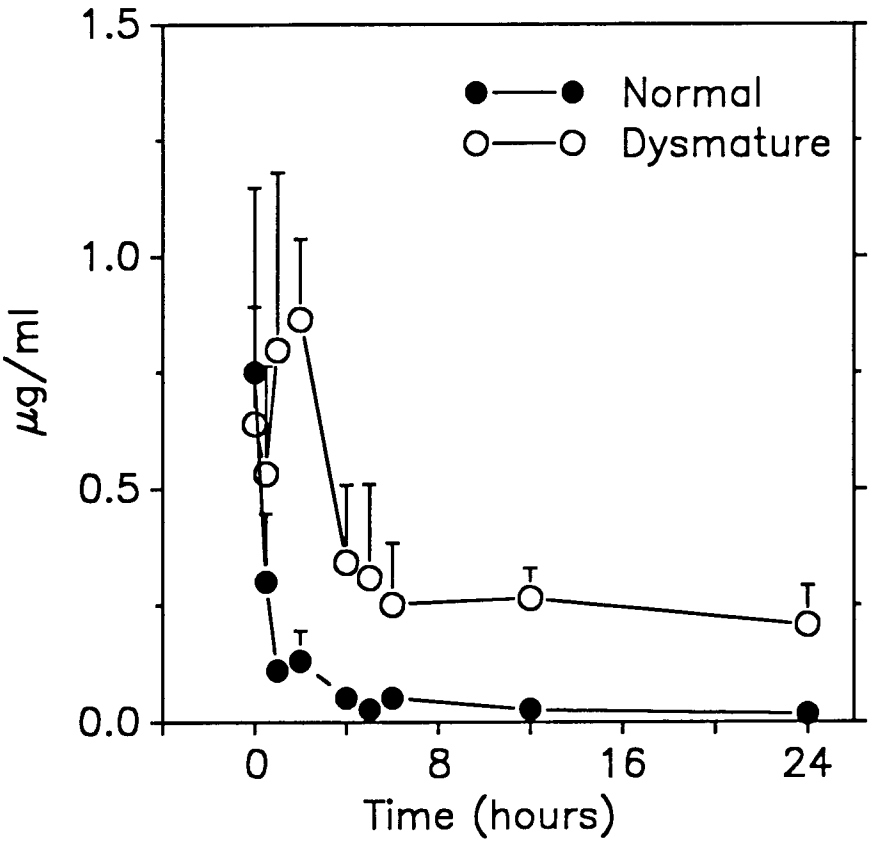

Figure 8. Plasma 5α-Pregnane-3ß,20α-diol (mean ± SE) in normal (n = 5) and dysmature (n = 3) newborn lighthorse and pony foals.

Table 9. Plasma 5α-pregnane-3ß,20α-diol (μg/ml, mean ± SE) in normal (n = 5) and dysmature (n = 3) newborn lighthorse and pony foals from birth (time 0) through 96 h of age.

Time	Normals	Dysmature
0	.75 ± .40[a]	.64 ± .25[a]
.5	.30 ± .14[a]	.53 ± .23[a]
1	.11 ± .01[a]	.80 ± .38[a]
2	.13 ± .06[a]	.86 ± .17[b]
4	.05 ± .01[a]	.34 ± .17[a]
5	.03 ± .01[¶]	.31 ± .20[¶]
6	.05 ± .02[a]	.25 ± .13[a]
12	.03 ± .01[a]	.27 ± .07[b]
24	.02 ± .01[a]	.21 ± .09[b]
48	BDL	.14 ± .04[¶]
72	BDL	.04 ± .03[¶]
96	BDL	BDL

[a,b] Values within a row followed by a different superscript are different ($P < .05$).
[¶] $n \leq 2$, not tested.
BDL Below detection limit of assay.

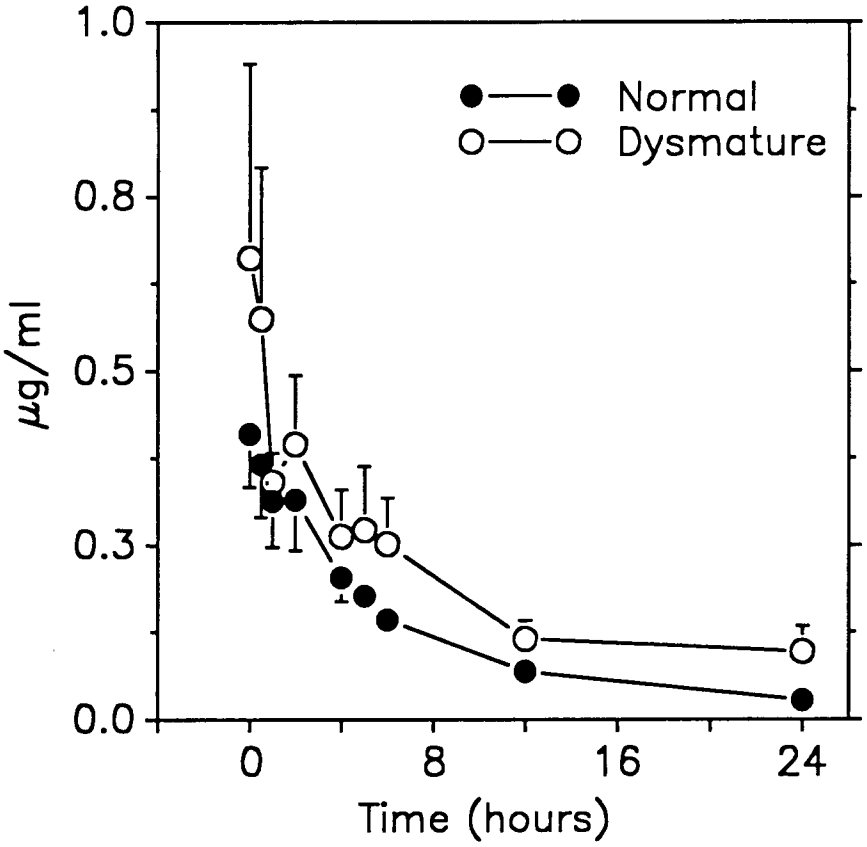

Figure 9. Plasma 3ß-Hydroxy-5α-pregnan-20-one (mean ± SE) in normal (n = 5) and dysmature (n = 3) newborn lighthorse and pony foals.

Table 10. Plasma 3ß-Hydroxy-5α-pregnan-20-one (μg/ml, mean ± SE) in normal (n = 5) and dysmature (n = 3) newborn lighthorse and pony foals from birth (time 0) through 96 h of age.

Time	Normals	Dysmature
0	.41 ± .07[a]	.66 ± .28[a]
.5	.37 ± .08[a]	.57 ± .22[a]
1	.31 ± .07[a]	.34 ± .04[a]
2	.32 ± .07[a]	.40 ± .10[a]
4	.20 ± .03[a]	.26 ± .07[a]
5	.18 ± .01[¶]	.27 ± .09[¶]
6	.14 ± .01[a]	.25 ± .07[a]
12	.07 ± .02[a]	.12 ± .03[a]
24	.03 ± .01[a]	.10 ± .03[a]
48	BDL	.20 ± .12[¶]
72	BDL	.38 ± .34[¶]
96	BDL	.25 ± .24[¶]

[a,b] Values within a row followed by a different superscript are different ($P < .05$).
[¶] $n \leq 2$, not tested.
BDL Below detection limit of assay.

Table 11. Plasma 20ß-hydroxy-4-pregnen-3-one concentrations (µg/ml, mean ± SE) in normal (n = 5) and dysmature (n = 3) newborn lighthorse and pony foals from birth (time 0) through 96 h of age.

Time	Normals	Dysmature
0	BDL	BDL
.5	BDL	BDL
1	.03 ± .01	BDL
2	---	.10 ± .04
4	.04 ± .01	.20 ± .14
5	.03 ± .01	.17 ± .09
6	.07 ± .04	.13 ± .08
12	---	---
24	BDL	.38 ± .03
48	BDL	.31 ± .04
72	BDL	.28 ± .25
96	BDL	---

DISCUSSION AND CONCLUSIONS

This study reported for the first time identification and quantification of a series of pregnenes and pregnanes in plasma from both normal and dysmature newborn foals. Pregnenolone, P5ßß, P5ßα, 5α-DHP-ßß, 5α-DHP-ßα and 3ß-OH-5α-DHP were detectable by GC/MS in elevated concentrations (μg/ml) which were comparable in type to those found in fetal and umbilical cord blood (Holtan et al., 1991).

Previously reported data (P5 and P5ßß) from this study (Houghton et al., 1991; Voller et al., 1991), as well as work presented here demonstrates that P5, 5α-DHP-ßß, 5α-DHP-ßα and 3ß-OH-5α-DHP exhibit maximum plasma concentrations at birth and clear rapidly from circulation within 48 h in normal foals. Pregnene-diols (P5ßß and P5ßα) are detectable at maximum levels later (4 h and 2 h, respectively), but also clear rapidly.

In contrast, dysmature foals demonstrate lower P5, P5ßß, P5ßα, 5α-DHP-ßß and 5α-DHP-ßα concentrations at birth thereafter increasing with a prolonged period of detection (> 48 h). Progestins, especially 20ß-hydroxylated compounds, tend to be elevated when compared to normal foals at 12 h through 48 h of age.

Plasma cortisol levels in normal and dysmature foals are in agreement with previous work (Rossdale et al.,

1982; Silver et al., 1984) and were used for initial identification of dysmaturity. Cortisol is maximum within 2 h of birth in normal foals and response to exogenous ACTH (.2 IU/kg BW, i.v., 4 h) is similar to that seen in the adult horse (James et al., 1970). Dysmature foals demonstrate lower cortisol levels at birth (25.17 ± 8.66 vs 99.54 ± 7.44 ng/ml, dysmature vs normal foals) and little or no response to exogenous ACTH. Cortisol levels also tended to be higher in dysmature foals when compared to normal foals from 12 h of age.

Abnormally low plasma cortisol, such as is seen at birth in dysmature foals, may indicate a depressed prepartum cortisol surge. The surge in plasma cortisol observed just prior to parturition in the fetus appears to be essential to organ maturation (lung, liver and gut), as well as activation of placental enzyme systems that are important to the initiation of parturition itself (Anderson et al., 1975). Aberrations in this surge may result in delayed or incomplete development of major organ systems and result in an individual ill equipped to normally adapt to an extrauterine environment. The elevated cortisol and progestin levels (> 48 h) seen in dysmature foals in this study could be a reflection of faulty steroid metabolism or reduced clearance due to

impaired function (possibly as a result of immaturity) of the liver or kidney.

Aberrant glucocorticoid levels may also point to abnormalities at the adrenal level. Lack of response to ACTH may suggest an inadequate level of adrenal maturity as the fetal adrenal does not respond to endogenous ACTH until close to term (Silver et al., 1984). Histologically, the fetal adrenal markedly differs from the adult adrenal gland in that the fetal cortex is much more pronounced (Benner, 1940). Naeye and co-workers (1971) demonstrated that adrenal glands were 19% lighter in human infants with hyaline membrane disease than in those with normal lung maturity due to the greater number of adrenal cortical cells (fetal and permanent) in the normal infants. This may limit the availability of cortical cells for steroid synthesis in dysmature or premature foals as reflected by the abnormal steroid profiles in the present study.

The absence of response to ACTH may also suggest a deficiency or inadequacy on an enzymatic level. Activity of 3ß-HSD, the key enzyme in the conversion of pregnenolone to progesterone, in the fetal adrenal increases significantly during late gestation (Sholl, 1983). The increase in plasma cortisol closely follows the increase in 3ß-HSD activity in the fetal adrenal

cortex. In the adult, inhibition of 3ß-HSD activity results in elevated pregnenolone and reduced plasma glucocorticoids (Potts et al., 1978). It is plausible that blockage or inactivity of this enzyme could result in similar steroid profiles in the dysmature or premature foal. In addition, 3ß-HSD activity may be further altered due to inhibitory effects of resulting steroids and(or) steroid metabolites (Hirato et al., 1982). This may explain the elevated levels of progestins and progestin metabolites in dysmature foals in this study as metabolism to cortisol is blocked early on.

In the present study a 3ß-HSD blocker, trilostane, was used to attempt to selectively suppress 3ß-HSD activity (Potts et al., 1978; Hiwatashi et al., 1985). Lack of response in cortisol and progestin profiles to blockage of 3ß-HSD by trilostane in this study may be due to several factors. Incomplete drug absorption by the foal may have resulted in sub-pharmacological doses. Other factors may have been related to inadequate dosage and(or) an overriding effect of the ACTH that was administered simultaneously.

Another aspect of the apparent inability of trilostane to suppress cortisol production may have been due to the chronological order of ACTH and trilostane treatments. It is possible that, although adequate levels

of trilostane had been attained in the blood, the pharmacologic peak was reached sometime after the effect of ACTH. Due to limitations as to the route of administration for trilostane (oral) and lack of information as to the pharmacokinetics of the drug in the horse, this could not be addressed directly.

Fowden et al. (1987) found that the administration of related 3ß-HSD blocker (epostane) did not induce parturition in the mare as it did in the ewe, although there was a significant reduction in immune-reactive progesterone in the mare. This suggests a species difference in the steroidogenic pathway, which may offer another explanation as to the ineffectiveness of trilostane in this study to inhibit glucocorticoid production in the foal.

The effect of elevated cortisol and progestin levels in dysmature foals is unknown. However, typically these animals demonstrate slower responses to environmental stimuli and often exhibit other signs normally attributed to prematurity (e.g. slow suck reflex, inability to stand, hypoflexion of the pasterns). Although glucocorticoids, such as cortisol, are involved in the stress response (Martin, 1985), it may also be of interest that certain steroids, particularly 5α- or 5ß-reduced pregnanes may bind with inhibitory neurotransmitter receptors, such as

GABA (Harrison et al., 1987; Peters et al., 1988). In mammals, the central nervous system contains unusually high concentrations of several amino acids, such as GABA, that have the ability to alter neuronal discharge (Goodman and Gillman, 1987). One study indicated that a progesterone metabolite, 5α-pregnane-3α-hydroxy-20-one possessed GABA-like activity (Harrision et al., 1987). Later work by Turner et al., (1989) demonstrated that modulation of the GABA-receptor complex by steroid anesthetics and naturally occurring analogs was at a site distinct from barbiturates. Progesterone metabolites have also been shown to be potent inhibitors of uterine contractility through GABA (Putman et al., 1991). It is conceivable that pathologically high levels of progestin metabolites such as those found in this study may be responsible for an anesthetic effect by interaction with GABA receptor systems. This may be in part, an explanation of the lack of responsiveness and prolonged lethargy often seen in premature or dysmature foals.

Both P5ßß and P5ßα (pregnenolone metabolites) were found at significantly elevated levels in dysmature foals when compared to normal foals over the 96 h study period. However, plasma concentrations of P5ßß were approximately 5 to 10 times higher than P5ßα. Similar profiles are seen when comparing plasma concentrations of 5α-DHP-ßß to 5α-

DHP-ßα; 5α-DHP-ßß concentrations are consistently (2 to 3 times) higher than 5α-DHP-ßα over time. Although similar contrasts are seen in normal foals (3ß,20ß-hydroxylates are found in higher concentrations), all progestins are below assay detection limits by 72 to 96 h. In dysmature foals, plasma levels of progestins were still elevated at 96 h.

Previous work by Holtan and co-workers (1991) showed the fetus to be the primary source of 3ß- and 20ß-hydroxylated compounds. Data from this study provides additional information in regard to metabolites and metabolic profiles of several steroids previously not examined in the equine neonate. Results suggest a metabolic preference for 3ß,20ß-hydroxylates in the feto-placental unit. Parturition eliminates the influence of the placenta as a source of steroid metabolism, which may explain in part the rapid decrease in progestin concentrations seen in normal foals. This suggests that the elevated levels of most progestins seen in dysmature foals after 12 h in this study could be related to a premature separation of the feto-placental unit as the dysmature foal may not be fully capable of metabolizing these progestins without assistance from placental enzyme systems due to lack of hepatic and(or) renal maturity.

No progesterone was found in either normal or dysmature foals via GC/MS analysis. However, samples tested from three foals by commercial RIA (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA) indicated immuno-reactive progesterone in high levels (> 20 ng/ml) at birth. Discrepancies in results between these two methods may be explained by correlating the elevated levels of endogenous progestins (μg/ml) with the crossreactivity of the antiserum against progesterone utilized by the RIA. Under most conditions, lack of or low levels of crossreactivity of an antiserum at equal concentrations is an adequate indicator of the specificity of an immunoassay. However, if an interfering steroid is found in 10- to 100-fold concentrations in relation to the hormone tested, as in these studies, even low crossreactivities may become significant. GC/MS analysis avoids the problem of crossreactivity as well as providing accurate identification of closely related compounds through mass-ion chromatographs. This proved to be invaluable in this study where several of the compounds measured were stereoisomers.

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APPENDICES

Appendix A: Plasma steroid concentrations for individual newborn lighthorse and pony foals.

Table A1. Plasma cortisol concentrations (ng/ml) in normal (Mayday, Jeana, Bing and Slopes) and dysmature (PF#43, PF#44 and Amy) foals from birth (time 0) through 96 h of age.

Time	Mayday	Jeana	Bing	Slopes	PF#43	PF#44	Amy
0	83.58	99.64	86.19		42.22	13.96	19.33
.5	105.60	76.96	83.12		34.37	24.05	25.84
1	50.55				38.69	25.60	32.17
2	17.13	118.36	52.84		52.26	26.88	33.83
4		33.09	58.47	21.48	63.02	22.47	33.67
4.5					63.97	28.50	30.61
5			38.76		63.93	31.66	31.02
6	19.25	28.67		24.40	67.55	30.35	31.15
8				10.87	56.00	21.58	32.23
12	9.48	23.84	5.87	27.09	51.38	17.31	47.51
16	16.44				21.96	15.33	
24	6.43	16.07	8.26	13.78	18.93	13.29	67.17
48	5.13	17.39	6.15	12.00	29.98	14.23	51.73
72	3.23	8.17	8.39	12.86	18.66	12.53	
96	5.62	20.73	5.56	7.74	7.95	21.33	

Table A2. Plasma cortisol concentrations (ng/ml) in ACTH treated (.2 IU/kg, BW, 4 h) foals from birth (time 0) through 96 h of age.

Time	PF#35[1]	PF#60[1]	Gypsy[1]	Pokey[1]	Irene[1]	PF#56	PF#46	PF#48
0	103.15					133.40	91.25	
.5	169.15					175.78	122.67	
1	113.20					145.31	187.64	
2	120.46	18.31		13.16		149.50	118.82	
4	51.89	13.67	14.23	32.60	14.11	58.58	51.30	21.40
4.5	105.45	95.80	74.38	101.36	94.68	148.78	91.81	95.69
5	164.46	119.29	120.48	126.66	106.86	188.53	125.79	109.20
6	152.57	176.13	134.55	121.58	55.76	104.61	175.96	111.16
8	53.16	41.60	73.41	32.29	17.72	58.87	97.75	27.37
12	14.36	7.95	3.03	12.60	8.08	27.67	32.47	8.03
16	8.12	4.32		25.90	5.93	19.13	14.59	6.35
24	14.70	6.36	10.98	18.52	4.79	5.44	9.30	4.78
48	4.27	12.02	3.61	13.92	13.20	5.75	3.26	15.28
72	8.63	3.70	6.13	15.27	10.21	6.12	4.72	13.25
96	2.46	9.06	16.10	10.36	4.09	10.48	9.19	13.02

[1] Foals also treated with trilostane at 4 h (10 mg/kg, p.o.) ($P > .05$).

Table A3. Plasma 3ß-hydroxy-5-pregnen-20-one concentrations (µg/ml) in normal (Mayday, Jeana, Bing, PF#35 and PF#56) and dysmature (PF#43, PF#44 and Amy) foals from birth (time 0) through 96 h of age.

Time	MayDay	Jeana	Bing	PF#35	PF#56	PF#43	PF#44	Amy
0	1.60	3.39	1.23	2.67	2.69	.67	1.90	.82
.5	1.73	2.56	1.17	2.31	2.83	.98	5.07	1.12
1	1.35			2.20	2.68	1.00	4.23	1.26
2	.88	3.05	1.12	1.80	2.16	1.21	3.40	1.36
4		2.07	1.32	1.18	1.36	1.15	2.27	.94
5				1.02	1.20	1.09	2.23	
6	.79	1.65	1.26	.84	1.10	.90	1.81	.95
12	.02	1.36	.25	.38	1.39	.51	1.24	.99
24	.18	.78	.24	.30	.45	.76	1.93	.42
48	.07	.06	.07	.06	.06	1.33	4.69	.96
72	.03	.02	.03			.47	4.89	
96						.07	2.10	

Table A4. Plasma 5-pregnene-3ß,20ß-diol concentrations (µg/ml) in normal (Mayday, Jeana, Bing, PF#35 and PF#56) and dysmature (PF#43, PF#44 and Amy) foals from birth (time 0) through 96 h of age.

Time	MayDay	Jeana	Bing	PF#35	PF#56	PF#43	PF#44	Amy
0	1.14	6.19	4.81	1.91	1.39	1.14	.65	1.10
.5	3.34	2.90	4.13	3.23	2.57	3.86	10.28	3.62
1	4.45			3.99	3.37	5.59	10.04	6.73
2	5.80	7.15	5.56	4.80	3.76	6.34	12.30	11.98
4		9.95	6.78	5.01	3.47	9.77	13.54	12.46
5				4.16	3.29	9.01	13.48	
6	4.23	8.09	6.74	4.13	2.98	10.14	16.47	12.91
12	1.03	7.25	1.26	1.87	2.83	9.01	16.43	14.48
24	.77	3.54	.82	.90	.81	10.23	21.99	12.51
48	.52	.26	.24	.21	.22	9.19	30.77	8.69
72	.11	.07	.09			2.03	24.93	
96						.21	8.84	

Table A5. Plasma 5-pregnene-3ß,20α-diol concentrations (μg/ml) in normal (Mayday, Jeana, Bing, PF#35 and PF#56) and dysmature (PF#43, PF#44 and Amy) foals from birth (time 0) through 96 h of age.

Time	MayDay	Jeana	Bing	PF#35	PF#56	PF#43	PF#44	Amy
0	.24	.84	.43	.43	.37	.13	.14	.18
.5	.45	.49	.39	.46	.39	.26	1.21	.49
1	.42			.47	.43	.37	.84	.71
2		.79	.43	.44	.39	.51	1.05	.98
4		.65	.47	.29	.19	.52	.79	.75
5				.19	.17	.66	.76	
6	.22	.43	.48	.17	.13	.58	1.07	.89
12	.07	.28	.09	.07	.16	.48	1.09	1.14
24	.05	.17	.09		.05	.47	1.05	1.15
48	.03		.03		.02	.27	1.32	.65
72						.13	.83	
96							.33	

Table A6. Plasma 5α-pregnane-3ß,20ß-diol concentrations (μg/ml) in normal (Mayday, Jeana, Bing, PF#35 and PF#56) and dysmature (PF#43, PF#44 and Amy) foals from birth (time 0) through 96 h of age.

Time	MayDay	Jeana	Bing	PF#35	PF#56	PF#43	PF#44	Amy
0	.35	3.86	.57	.52	.51	.70	.95	.67
.5	.53	1.21	.58	.47	.54	.83	1.62	1.01
1	.58			.56	.55	.87	3.67	2.32
2	.47	1.21	.58	.59	.61	.88	2.90	3.21
4		1.30	.58	.61	.60	1.52	3.79	2.26
5				.50	.51	1.43	3.75	
6	.46	2.95	.55	.32	.44	1.11	5.00	1.00
12	.09	1.61	.23	.16	.22	2.08	3.59	4.56
24	.03	.73	.03		.05	3.15	3.64	3.15
48	.02				.01	.73	3.77	2.62
72						.19	4.05	
96							2.36	

Table A7. Plasma 5α-pregnane-3ß,20α-diol concentrations (μg/ml) in normal (Mayday, Jeana, Bing, PF#35 and PF#56) and dysmature (PF#43, PF#44 and Amy) foals from birth (time 0) through 96 h of age.

Time	MayDay	Jeana	Bing	PF#35	PF#56	PF#43	PF#44	Amy
0	.37	1.94		.34	.35	.17	.73	1.03
.5	.20	.87	.08	.17	.18	.10	.60	.89
1	.10			.12	.11	.10	.88	1.41
2		.32	.05	.07	.08		.69	1.04
4		.09	.04	.04	.03	.08	.64	.31
5				.03	.03	.11	.51	
6	.03	.15	.04	.02	.02	.09	.51	.15
12	.01	.06	.03		.01	.15	.37	.28
24	.01	.05	.01		.01	.10	.15	.38
48							.10	.19
72						.01	.08	
96							.06	

Table A8. Plasma 3ß-hydroxy-5α-pregnan-20-one concentrations (μg/ml) in normal (Mayday, Jeana, Bing, PF#35 and PF#56) and dysmature (PF#43, PF#44 and Amy) foals from birth (time 0) through 96 h of age.

Time	MayDay	Jeana	Bing	PF#35	PF#56	PF#43	PF#44	Amy
0	.28	.53	.20	.42	.62	.50	1.20	.28
.5	.24	.57	.17	.35	.50	.42	1.00	.30
1	.20			.31	.43	.33	.42	.28
2		.47	.14	.26	.39	.40	.56	.22
4		.27	.11	.19	.23	.27	.37	.14
5				.17	.18	.18	.36	
6	.11	.17	.11	.15	.18	.28	.35	.13
12	.03	.13	.03	.04	.11	.08	.17	.09
24	.01	.05	.01		.04	.08	.17	.04
48					.004	.11	.43	.06
72						.04	.73	
96						.01	.49	

Table A9. Plasma 20ß-hydroxy-4-pregnen-3-one concentrations (µg/ml) in normal (Mayday, Jeana, Bing, PF#35 and PF#56) and dysmature (PF#43, PF#44 and Amy) foals from birth (time 0) through 96 h of age.

Time	MayDay	Jeana	Bing	PF#35	PF#56	PF#43	PF#44	Amy
0								
.5								
1	.02				.04			
2					.03		.14	.07
4		.06	.04		.02		.34	.06
5	.02				.03	.08	.25	
6		.15	.03		.02		.22	.05
12					.04		.31	
24						.40	.43	.33
48						.27	.39	.27
72						.03	.54	
96							.25	

Appendix B. Total ion chromatographs and full-scans of selected progestins and deuterium-labelled progestin standards.

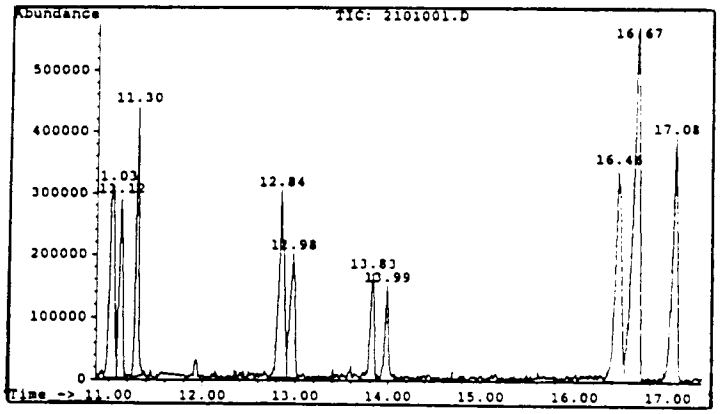

Figure A1. Total ion chromatograph of progestin standards on GC/MS full scan mode. Major peaks are: doublet 5α-pregnene-3,20-dione (11.30 and 11.12 min), P4 (11.30 min), P5 (12.84 min), 3ß-OH-5α-DHP (12.98 min), 20α-OH-5α-DHP (13.83 and 13.99), 20ß-OH-4-pregnen-3-one (approximately 13.90 min, not shown), P5ßß (16.49 min), 5α-DHP-ßß (16.67) and 5α-DHP-ßα (17.08 min). Horizontal axis refers to retention time, vertical axis refers to abundance of ions.

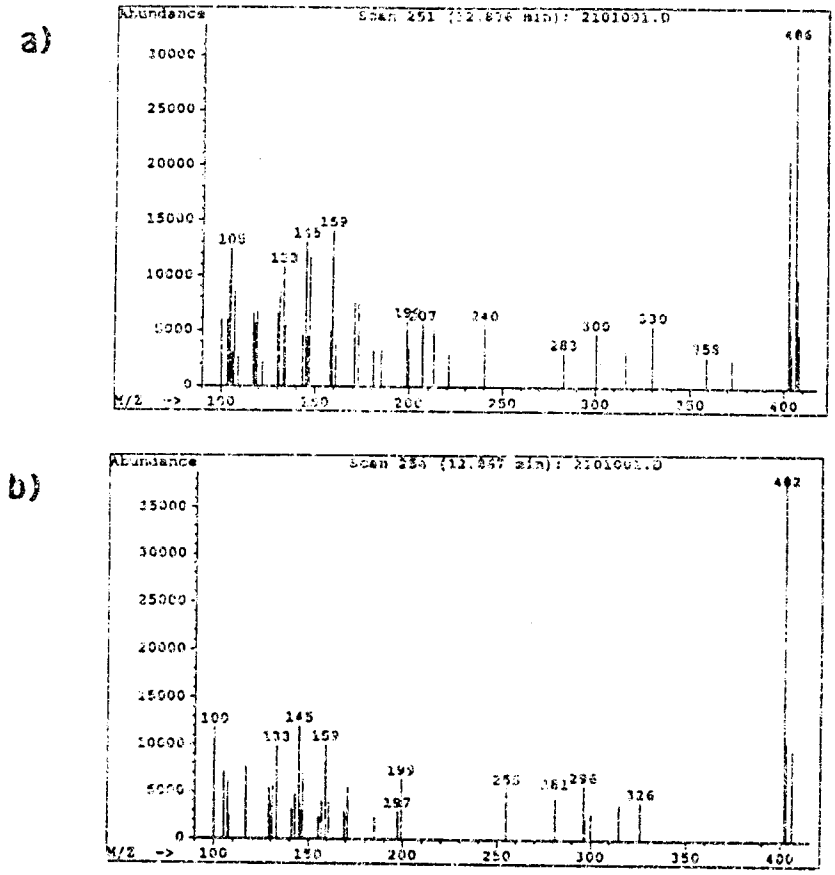

Figure A2. Full scans of a) D_4-P5 and b) P5. Pregnenolone is quantified against D_4-P5 with major ions at 406 and 402 respectively. Mass to charge ratio (m/z) is on the horizontal axis, abundance of ions on the vertical axis.

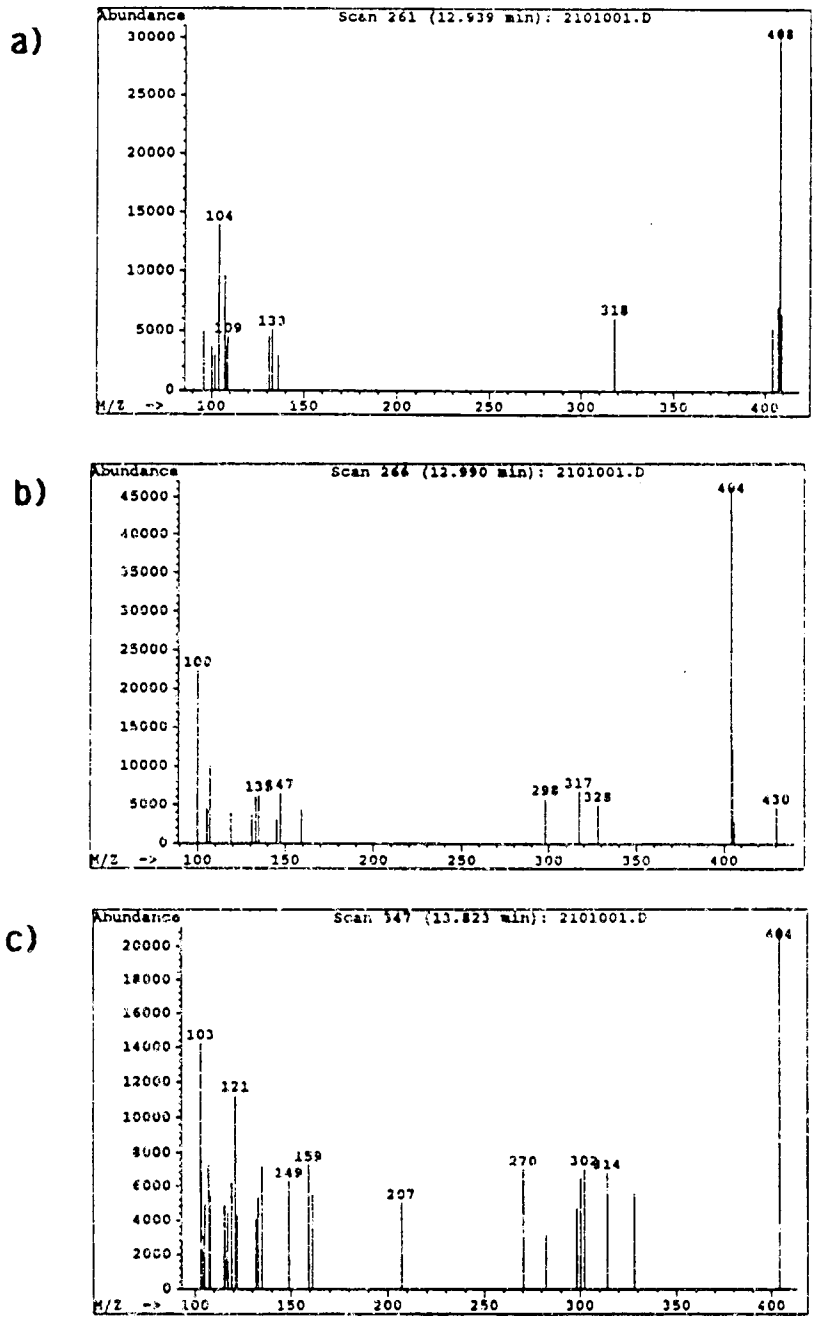

Figure A3. Full scans of a) D_4-3ß-OH-5α-DHP, b) 3ß-OH-5α-DHP and c) 20α-OH-5α-DHP. 3ß-OH-5α-DHP is quantified against D_4-3ß-OH-5α-DHP with major ions at 408 and 404, respectively. Mass to charge ratio (m/z) is on the horizontal axis, abundance of ions on the vertical axis.

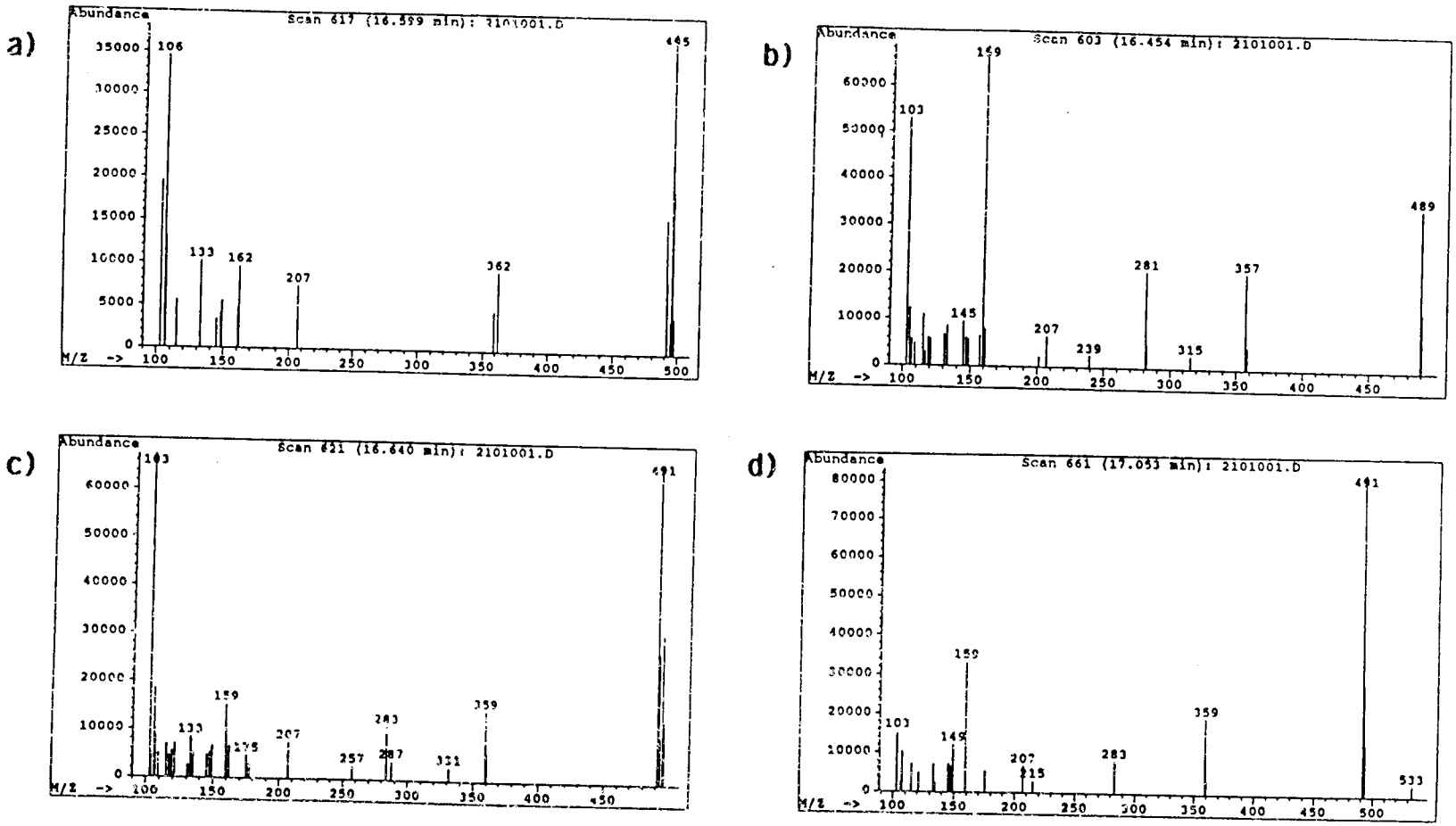

Figure A4. Full scans of a) D_4-5α-DHP-ββ, b) P5ββ, c) 5α-DHP-ββ and d) 5α-DHP-βα. Major ions monitored are 495 for D_4-5α-DHP-ββ, 489 for P5ββ and 491 for both 5α-DHP-ββ and 5α-DHP-βα. All three diols are quantified with D_4-5α-DHP-ββ. Mass to charge ratio (m/z) is on the horizontal axis, abundance of ions on the vertical axis.