Differential effect of trilostane on the progestin milieu in the pregnant mare

W. E. Schutzer^{1*}, J. L. Kerby² and D. W. Holtan^{1†}

¹Department of Animal Sciences, and ²College of Pharmacy, Oregon State University, Corvallis, OR 97331, USA

Trilostane, a competitive inhibitor of 3 β -hydroxysteroid dehydrogenase, was administered intravenously to pregnant mares ($n = 3$) between day 277 and day 282 of gestation to determine its effect on the progestin milieu. In addition, placental tissue from mares at mid-gestation (150–300 days) ($n = 4$) were exposed to either deuterium-labelled pregnenolone alone or deuterium-labelled pregnenolone and trilostane to examine the effect of trilostane on placental metabolism of pregnenolone. Blood samples were collected from indwelling jugular catheters at frequent intervals for 48 h after infusion. Both plasma samples and incubation media were quantitatively analysed, after solid phase extraction and steroid derivitization, for concentrations of eight different progestin metabolites using gas chromatography and mass spectrometry. Trilostane infusion differentially affected the progestin milieu *in vivo* without inducing abortion. Forty-five minutes after infusion, maternal plasma concentrations of pregnenolone, 5-pregnene-3 β ,20 β -diol, 5 α -pregnane-3 β ,20 β -diol and 3 β -hydroxy-5 α -pregnan-20-one increased ($P < 0.05$) and remained high for 37 h. Concentrations of 5 α -pregnane-3,20-dione, 20 α -hydroxy-5 α -pregnan-3-one and 5 α -pregnane-3 β ,20 α -diol decreased 15 min after infusion ($P < 0.05$), yet 1.5 h after infusion, concentrations had increased and remained high until 37 h after infusion. Trilostane inhibited the conversion of pregnenolone to progesterone *in vitro* ($P < 0.001$) while mediating an increase ($P < 0.05$) in concentrations of 5 α -pregnane-3,20-dione and 3 β -hydroxy-5 α -pregnan-20-one. These observations demonstrate that the pregnant mare possesses unique steroid hormone metabolic activity and suggests that another steroid, perhaps 5 α -pregnane-3,20-dione and not progesterone, is the important steroid precursor for the other progestin metabolites found in circulating plasma.

Introduction

The mare possesses unique steroid hormone metabolic activity during mid- and late-gestation. In contrast to other mammals, where circulating progesterone concentrations are generally raised throughout pregnancy, equine maternal concentrations of progesterone begin to decrease at about day 125 of gestation and are undetectable, or very low, near day 200 of gestation (term = 340 days). Furthermore, eight distinct, prominent progestin metabolites have been identified throughout pregnancy in plasma from pregnant mares (Holtan *et al.*, 1991). In addition, the mare is unusual in that ovariectomy as early as at day 50 of gestation has no adverse effect on pregnancy maintenance (Holtan *et al.*, 1979; Knowles *et al.*, 1994) indicating that pregnancy is maintained henceforth by an extra-luteal source of progestin. One progestin metabolite, 5 α -pregnane-3,20-dione, (5 α -DHP), is detectable in the peripheral circulation as early as day 10 (Atkins *et al.*, 1976) and

concentrations of other metabolites increase throughout pregnancy (Holtan *et al.*, 1975). Another progestin metabolite, 20 α -hydroxy-5 α -pregnan-3-one (20 α -5 α), as identified with gas chromatography/mass spectrometry (GC/MS), can reach concentrations of $\mu\text{g ml}^{-1}$ near parturition, (Holtan *et al.*, 1991). The systematic names, abbreviations and structural details of the progestins that are discussed throughout this text, are given in Table 1.

In common with other species, the equine placenta contains 3 β -hydroxysteroid-dehydrogenase (3 β -HSD) activity; the placenta metabolizes pregnenolone to progesterone (Ainsworth and Ryan, 1969; Schutzer and Holtan, 1996). It has thus been suggested that maintenance of pregnancy in mares is controlled primarily by progestins of a placental source (Moss *et al.*, 1979). To examine this possibility, Fowden and Silver (1987) examined the effect of epostane, a 3 β -HSD competitive inhibitor, on plasma progesterone concentrations and on the duration of gestation in mares near term. Although treatment decreased immunoreactive-progesterone, it did not cause abortion. In contrast, abortion was induced when pregnant rhesus monkeys, rats, sheep, swine, cows or humans were exposed to similar or lower doses of a 3 β -HSD competitive

*Current address: Division of Reproductive Sciences, Oregon Regional Primate Research Center, Beaverton, OR 97006, USA.

†Correspondence.

Received 5 January 1996.

Table 1. Systematic names, abbreviations and structural details of steroids

Systematic name	Abbreviation	R1	R2
5-Pregnenes			
3 β -hydroxy-5-pregnen-20-one	Pregnenolone	β -OH	= O
5-pregnen-3 β ,20 β -diol	Pregnenolone-5- $\beta\beta$	β -OH	β -OH
4-Pregnenes			
4-pregnen-3,20-dione	Progesterone	= O	= O
5 α -Pregnanes			
5 α -pregnane-3,20-dione	5 α -DHP	= O	= O
3 β -hydroxy-5 α -pregnan-20-one	3 β -5 α	β -OH	= O
20 α -hydroxy-5 α -pregnan-3-one	20 α -5 α	= O	α -OH
5 α -pregnane-3 β ,20 β -diol	$\beta\beta$ -diol	β -OH	β -OH
5 α -pregnane-3 β ,20 α -diol	$\beta\alpha$ -diol	β -OH	α -OH

inhibitor *in vivo* (Schane *et al.*, 1979; Creange *et al.*, 1981; Taylor *et al.*, 1982; Martin *et al.*, 1987; Crooij *et al.*, 1988). Likewise, inhibition of 3 β -HSD *in vitro* stopped the conversion of pregnenolone to progesterone in dispersed sheep placental cells (Power and Challis, 1987).

It is important to recognize that Fowden and Silver (1987) measured immunoreactive progestins and not progesterone itself; all of the progestin metabolites crossreact to some extent with 'progesterone-specific' antibodies in radioimmunoassays. Therefore, in this study, which examined how inhibition of 3 β -HSD affects individual progestins, GC/MS was applied which measures quantitatively concentrations of each specific progestin metabolite.

Trilostane, a 3 β -HSD competitive inhibitor similar in function to epostane (Potts *et al.*, 1978; Ledger *et al.*, 1985), was infused intravenously into mares ($n = 3$) at mid-gestation to examine its effect on the progestin milieu. By examining trilostane-mediated alterations of individual progestin concentrations, metabolic activity, biological relevance and steroidal precursors of the progestins found in maternal circulation can be established. The specific role of the placenta in steroid metabolism, and therefore pregnancy maintenance, was also investigated by using trilostane *in vitro*.

Materials and Methods

Animals

Three pregnant pony mares (*Equus caballus*; approximately 200 kg) of unknown pedigree were maintained in outdoor paddocks and fed a ration which met appropriate husbandry standards. The duration of gestation of the mares at the time of infusion was: 282 days, 280 days, and 277 days. The animals in the *in vivo* study had been used previously in a similar study (see Schutzer and Holtan (1996) for details).

Experimental procedures *in vivo*

Mares ($n = 3$) received 5 g 3 β -HSD competitive inhibitor, trilostane ((4 α ,5 α ,17 β)-4,5-epoxy-3,17-dihydroxyandrost-2-ene-2-carbonitrile; WIN 24540; generously donated by Sterling

Winthrop Pharmaceuticals, Collegeville, PA) *i.v.* Trilostane was dissolved (90 mg ml⁻¹) in dimethyl sulfoxide (Sigma, St Louis, MO) and then this solution was added to a mixture made up of 75% (v/v) propylene glycol (Sigma) and 5% (v/v) Tween-80 (Sigma). Seven days later, two of the mares received vehicle alone and served as controls. Catheters (16 gauge $\times$ 5.75 in; Angiocath; Desuet Medical, Sandy, UT) were placed into the jugular vein using an aseptic technique. Three blood samples were taken at intervals of 15 min. Over a 15 min period, trilostane was infused. Immediately after the infusion, blood samples were taken every 15 min for 2 h, every 30 min for 2 h, once an hour for 4 h and then every 12 h for 3 days. Blood was collected into heparinized tubes (Vacutainer; Becton Dickson, Rutherford, NJ) and immediately centrifuged at 1000 *g* for 15 min. Plasma was stored at -20°C until analysis.

Experimental procedures *in vitro*

Homogenous regions of placenta, without the chorionic girdle or endometrial cells, were collected at random from light horse mares ($n = 4$; approx. 500 kg) at a local abattoir. The gestation ages of the fetuses as estimated by measurement of the crown-rump length (Ginther, 1992) were 150 days, 250 days, 300 days and 320 days. The placentae were separated manually from the uteri and immediately placed on ice. After transport to the laboratory, 1 g portions of each placenta were finely minced and placed in sterile 20 ml glass tubes with rubber stoppers. Five ml of sterile filtered and gassed (95% O₂:5% CO₂) TCM-199 with Hank's salts and sodium bicarbonate (Sigma) were added. After suspension, the tubes were gassed, covered and mixed on their sides for 60 min at 37°C in an environmental shaker. After pre-incubation for 60 min, the tubes were centrifuged at 1000 *g* for 10 min and the medium poured off. Fresh gassed-medium (5 ml) was added; the tissue was resuspended and either 25 μg deuterium labelled (D4)-pregnenolone control or 25 μg D4-pregnenolone plus 2.0 μmol of trilostane in DMSO were added to the medium. The flasks were incubated and mixed at 37°C for 4 h on their side. After incubation, the tubes were centrifuged at 750 *g* at 5°C for 15 min and stored at -20°C until assay.

Progestin analysis

A Hewlett Packard (Wilmington, DE) 5890 GC interfaced with a 5971A MS was used for quantitative analysis on eight different endogenous progestins as described by Houghton *et al.* (1990) and verified by Holtan *et al.* (1991). Basic preparation involved internal standard fortification of plasma samples with D4-progestins (D4-5 α -DHP, D4-pregnenolone, D4-3 β -5 α and D4-5 α -pregnane-3 β ,20 β -diol (D4- $\beta\beta$ -diol)) prepared as described by Dehennin *et al.* (1980). The media samples used 4-cholesten-3-one as internal standard. Standard regression response lines were established for each steroid by adding increasing amounts of unlabelled standard (0–1000 ng) plus a constant amount (500 ng for D4-progestin or 2000 ng for 4-cholesten-3-one) of internal standard to either ovariectomized mare plasma or media.

Fortified samples were eluted through solid phase C-18 SepPaks (Millipore-Waters Chromatography, Marlborough, MA) and derivitized with a two-step process yielding methoxime or tert-butyldimethylsilyl derivatives or both. Ketone derivatives were produced with 2% (w/v) methoxyamine HCl (Sigma) solution in silation grade pyridine (Regis Chemical Company, Morton Grove, IL) while hydroxyl derivatives were produced with *n*-methyl-*n*-(*t*-butyldimethylsilyl)-trifluoroacetamide (Regis) in a catalytic solution of 5% (w/v) diethylamine (Sigma) in silation grade dimethylformamide (Regis). Derivatization was necessary to ensure steroid stability, improve chromatography, increase sensitivity and aid in identification. Samples were analysed by the temperature program mode in the gas chromatograph; mass spectrometry was then used for both identification (under full-scan mode) and quantitation (under selected ion mode). Positive identification of unknowns included 'fingerprints' of molecular ion fragmentation patterns compared with standards and relative retention times. Quantitation was accomplished by using least squares linear regression of standards versus their internal standards.

Statistical analysis

Absolute progestin concentrations from plasma were transformed to percentage change from first sample and then analysed with SAS (Version 6.62; SAS Inc., Cary, NC) under the General Linear Models Procedure for repeated measures. Deuterium-labelled steroid concentrations from incubation media were transformed to percentage conversion by dividing the concentration of each detected D4-metabolite by the total amount of D4-substrate initially added. Differences were compared with a two sample *t* test for means with Microsoft Excel for Windows (Version 5.0; Microsoft Corp., Seattle, WA).

Results

Experiments in vivo

Infusion of trilostane differentially affected progestin concentrations over 48 h (Fig. 1). However, progesterone was not detected in any samples throughout this study (sensitivity for progesterone was 500 pg ml⁻¹). There was an immediate

increase in the concentration of plasma pregnenolone, the direct substrate for 3 β -HSD (Fig. 2; inset). Likewise, concentrations of 5-pregnene-3 β ,20 β -diol (pregnenolone- $\beta\beta$), $\beta\beta$ -diol and 3 β -5 α also increased as a result of trilostane treatment (Fig. 2). However, concentrations of 5 α -DHP, 20 α -5 α , and 5 α -pregnane-3 β ,20 α -diol ($\beta\alpha$ -diol) initially decreased, but 2 h after the infusion had increased.

Average values for pregnenolone (Fig. 2; inset) increased nearly tenfold ($P < 0.001$) 30 min after the infusion, but steadily decreased over time until 37 h after infusion when no difference ($P > 0.05$) from pre-sampling was detected (Fig. 1). Since pregnenolone- $\beta\beta$ was not detected in plasma before the infusion (Fig. 2; inset), the effect of trilostane cannot be determined by comparison with pre-sample concentrations. However, immediately after infusion the average absolute concentration of pregnenolone- $\beta\beta$ in plasma was 24.9 ± 4.6 ng ml⁻¹ (Fig. 2), which steadily decreased over time until 5 h after infusion when plasma pregnenolone- $\beta\beta$ became undetectable (Fig. 1). Forty-five minutes after trilostane infusion, 3 β -5 α values were higher ($P < 0.05$) than pre-sample concentrations (Fig. 2) and remained raised up to 37 h after the infusion (Fig. 1). Values of $\beta\beta$ -diol were higher than pre-sample concentrations 30 min after the infusion (Fig. 2) and remained raised ($P < 0.05$) until 37 h after infusion (Fig. 1).

The changes in progestins 5 α -DHP, 20 α -5 α and $\beta\alpha$ -diol were all similar after treatment. The values reached an observed nadir ($P < 0.01$) 15 min after infusion (Fig. 2), but 60 min after infusion values were the same as pretreatment values ($P > 0.05$; Fig. 2). Six hours after infusion, 5 α -DHP values reached an observed vertex ($P = 0.01$) and 37 h after infusion were back to pretreatment values ($P > 0.05$; Fig. 1). Likewise 20 α -5 α values reached an observed apex 6 h after infusion ($P = 0.0002$; Fig. 2) and then decreased (Fig. 1). Values for $\beta\alpha$ -diol reached an observed maximum 3 h after infusion ($P = 0.009$; Fig. 2) and then decreased.

There was no effect ($P > 0.1$; data not shown) of vehicle on the concentrations of any of the progestins in control mares. All three mares spontaneously delivered healthy foals within acceptable gestation periods.

Experiments in vitro

Trilostane significantly reduced ($P < 0.0001$) the placental conversion of D4-pregnenolone to D4-progesterone (Fig. 3) while stimulating an increase ($P < 0.05$) in the production of D4-5 α -DHP and D4-3 β -5 α . In D4-pregnenolone controls, $63.4 \pm 8.2\%$ of D4-pregnenolone was converted to D4-progesterone, while in the presence of trilostane, only $2.5 \pm 0.6\%$ was converted to D4-progesterone. Although only small amounts of D4-5 α -DHP ($0.2 \pm 0.1\%$) and D4-3 β -5 α ($1.5 \pm 0.5\%$) were detected in D4-pregnenolone control incubations, the increases seen after addition of trilostane represent 10- and 1.5-fold increases ($P < 0.05$), respectively, compared with controls.

Discussion

Trilostane infusion causes temporary inhibition of 3 β -HSD in the mid-gestation mare without inducing abortion. These

Fig. 1. Effect of trilostane on mean concentrations of circulating progestins in pregnant pony mares 4.5–45 h after treatment. Results are expressed in terms of the relative change from pretreatment samples. In the inset plot the relative changes are up to 30 times that of pretreatment values. Data points that are either above or below the broken line are significantly different ($P < 0.05$) from the first pretreatment sample. (○) 5 α -DHP; (●) 3 β -5 α ; (□) 20 α -5 α ; (■) $\beta\beta$ -diol; (△) $\beta\alpha$ -diol; (▲) pregnenolone; (◇) pregnenolone- $\beta\beta$. See Table 1 for abbreviations.

results are in agreement with those of Fowden and Silver (1987) who used epistane to inhibit 3 β -HSD activity. However, our results differ from those of Fowden and Silver (1987) in that they reported an epistane-mediated depression in progesterone while our results show a trilostane-mediated differential effect in the progestin milieu. This difference stems from the inability of radioimmunoassays to quantify different progestin metabolites; all 'progesterone-specific' assays cross-react with each of the progestin metabolites to some extent. Therefore, it is likely that Fowden and Silver (1987) were measuring a depression in total immunoreactive progestins and not in progesterone itself.

Rapid metabolism of pregnenolone to progesterone and the subsequent conversion of progesterone by the placenta to other metabolites, specifically to 5 α -DHP, can take place. It is well established that the equine placenta efficiently converts pregnenolone to progesterone *in vitro* (Ainsworth and Ryan, 1969; Schutzer and Holtan, 1996). However, documentation of conversion of progesterone to 5 α -DHP is less defined. Moss *et al.* (1979) considered the placenta or endometrium to be capable of efficiently converting progesterone to 5 α -DHP *in vivo* and Hamon *et al.* (1991) indicated that progesterone

may be directly converted to 5 α -DHP primarily in the endometrium, and Schutzer and Holtan (1996) found similar results using both *in vitro* and *in vivo* systems. Nevertheless, only relatively small amounts of progesterone are found in umbilical venous blood (flow from the placenta to the fetus) and none is found in the uterine vein during mid-gestation. Instead, the major steroidal constituent of umbilical venous blood identified from cannulated mid-gestation and parturient mares is 5 α -DHP (Holtan *et al.*, 1991).

In terms of steroid metabolism, it appears that the primary role of the fetus is to produce pregnenolone. Certainly the fetus is involved in some steroid metabolism, but there are only minor differences in the steroid profiles of the umbilical artery (fetus to placenta) versus the umbilical vein blood. Hamon *et al.* (1991) suggest that, in terms of progestin metabolism, the contribution of the fetus may be insignificant. The outstanding difference in umbilical artery blood, compared with that of the umbilical vein, is pregnenolone and pregnenolone- $\beta\beta$ are the major steroids and no, or very little, progesterone, 5 α -DHP and 20 α -5 α are detected (Holtan *et al.*, 1991). We therefore propose that the 5 α -reduction of progesterone to 5 α -DHP is directed by a 5 α -reductase, an enzyme probably located in the

Fig. 2. Effect of trilostane on mean concentrations of circulating progestins in pregnant pony mares during the first 3 h after treatment expressed in terms of relative change from pretreatment samples. In the inset plot relative changes are up to 30 times that of pretreatment values. Data points that are either above or below the broken line are significantly different ($P < 0.05$) from the first pretreatment sample. (○) 5 α -DHP; (●) 3 β -5 α ; (□) 20 α -5 α ; (■) $\beta\beta$ -diol; (△) $\beta\alpha$ -diol; (▲) pregnenolone; (◇) pregnenolone- $\beta\beta$. See Table 1 for abbreviations.

placenta, endometrium or in both locations that is yet to be characterized.

Silver (1994) suggested that 5 α -reductase could also metabolize pregnenolone directly to 3 β -5 α . However, empirical evidence for the conversion of pregnenolone to 3 β -5 α is circumstantial. When D4-pregnenolone is infused into pregnant mares, substantial quantities of D4-3 β -5 α are detected. Likewise, in placental tissue cultures incubated with D4-pregnenolone, one of the progestins found in quantity is D4-3 β -5 α . Finally, the presence of a placenta is necessary for the conversion of pregnenolone to 3 β -5 α ; when pregnenolone is infused into either pregnant or nonpregnant mares, no 3 β -5 α is detected in the nonpregnant animals, while there is an injection-mediated increase in 3 β -5 α in pregnant mares (Schutzer and Holtan, 1996). The pregnant mare also possesses other unusual metabolic processes. Schutzer and Holtan (1996) found that 3 β -5 α can be oxidized to 5 α -DHP *in vivo* and *in vitro*. Consequently, there seem to be two different metabolic pathways to produce 5 α -DHP – one using the classic Δ^5 - Δ^4 pathway, in which pregnenolone is converted to progesterone through 3 β -HSD, and the other using 5 α -reductase, in which pregnenolone is converted to 3 β -5 α . Both progesterone and 3 β -5 α may be directly metabolized to 5 α -DHP through

5 α -reductase or 3-oxidase, respectively. A proposed progestin metabolic pathway is presented (Fig. 4).

Results from trilostane treatment both *in vivo* and *in vitro* in this study support the presence of this alternative metabolic pathway. Although plasma concentrations of pregnenolone increase nearly ten times as a result of 3 β -HSD inhibition, results suggest differential metabolism of pregnenolone. Clearly, the immediate increase in plasma pregnenolone- $\beta\beta$ is a result of a 20 β -reductase activity on pregnenolone while the subsequent increase in plasma $\beta\beta$ -diol can be explained with 5 α -reductase activity on pregnenolone- $\beta\beta$. The increase in plasma 3 β -5 α can be explained by direct 5 α -reductase activity on pregnenolone itself. The decrease in plasma concentrations of 5 α -DHP additionally suggest that pregnenolone may indeed be converted to progesterone. Hence, progesterone could then be rapidly converted to 5 α -DHP. Progesterone then becomes an important, but not the only, source of 5 α -DHP. The concomitant significant fall in plasma concentrations of 20 α -5 α and $\beta\alpha$ -diol suggest that these compounds derive directly from 5 α -DHP; 5 α -DHP is converted to 20 α -5 α and 20 α -5 α is further metabolized to $\beta\alpha$ -diol.

The inversion from depression to increase in plasma 5 α -DHP, 20 α -5 α and $\beta\alpha$ -diol observed around 1.25 h after

Fig. 3. Effect of trilostane on mare placental tissue incubates *in vitro*. Data are presented as media content in terms of mean percentage ($\pm$ SEM) of deuterium-labelled (D4) pregnenolone, D4-progesterone, D4-5 α -DHP and D4-3 β -5 α . The inset plot gives the media content (percentage $\pm$ SEM) of D4-5 α -DHP and D4-3 β -5 α with the scale expanded. (■) D4-pregnenolone control; (□) D4-pregnenolone plus trilostane; * P < 0.05; ** P < 0.01; *** P < 0.0001 as compared with D4-pregnenolone control. See Table 1 for abbreviations.

infusion is probably due to the high concentrations of pregnenolone (still nearly ten times those before treatment) found in circulation. These high concentrations of pregnenolone might be used as a substrate for other metabolic compounds. Schutzer and Holtan (1996) infused D4-pregnenolone *in vivo* and saw similar metabolic changes.

Analysis of studies *in vitro* further supports the view that the proposed enzymes reside in placental tissue. Trilostane not only abates conversion (P < 0.0001) of pregnenolone to progesterone in placental incubates, but also mediates an increase (P < 0.05) in 3 β -5 α and 5 α -DHP. Therefore, inhibition of 3 β -HSD is not sufficient to eliminate the production of 5 α -DHP; it may still be produced through oxidation of 3 β -5 α .

The inability of trilostane to induce abortion may be explained by the unusual steroid hormone status of mares. Progesterone is not detected in maternal circulation, in uterine venous or in umbilical artery blood after 220 days of gestation, and 5 α -DHP is undetectable in the umbilical artery (Holtan *et al.*, 1991). It is possible that fetally synthesized progestins, which may not be reflected in maternal blood, locally affect the

uterus thereby maintaining pregnancy. However, trilostane can cross the placenta and enter the fetal circulation, consequently affecting fetal 3 β -HSD in the same manner as it affects maternal 3 β -HSD. Also, both Hamon *et al.* (1991) and Silver (1994) indicated that fetal tissues contributed little to progestin metabolism. Nonetheless, progesterone was never detected in maternal circulation throughout this study. Therefore, perhaps some other steroid, or unique metabolism of progestins may assist in pregnancy maintenance.

In addition to supplying information about metabolic processes, our data may provide inferential data about the steroidal control of pregnancy in mares. The unique manipulation of steroid metabolism may be able to support pregnancy, after placentation, without progesterone or 3 β -HSD. The mare may convert pregnenolone directly to 3 β -5 α , which could be subsequently metabolized to 5 α -DHP. It may then be 5 α -DHP that serves as an important substrate for further metabolism, if not the major steroid of pregnancy.

A more appropriate test of trilostane's ability to induce abortion in mares would be to deliver it chronically. However,

Fig. 4. Proposed metabolic pathway for progestin in mares through mid- and late-gestation. 3β-HSD: 3β-hydroxysteroid dehydrogenase.

the main goal of this experiment was not to examine abortion, but to examine steroidal alterations induced by trilostane. However, abortion was induced when pregnant rhesus monkeys, rats, sheep, swine, cows or humans were exposed to dosages similar to or lower than that used in this study with a 3β-HSD competitive inhibitor *in vivo* (Schane *et al.*, 1979; Creange *et al.*, 1981; Taylor *et al.*, 1982; Martin *et al.*, 1987; Crooij *et al.*, 1988). Therefore, 5α-DHP, and not progesterone, may be the important substrate for formation of the 5α-pregnanes found in maternal circulation and could prove to be the compound responsible for pregnancy maintenance. It was suggested that 5α-DHP was of some importance. Hamon *et al.* (1991) indicated that 5α-DHP could exert central effects in the fetus or mare, and Silver (1994) proposed that 5α-DHP could maintain the myometrium in a quiescent state and might therefore possess progestagenic bioactivity. The alternative pathway may alleviate the steroidal imbalance that trilostane creates. It is suggested that progestin metabolites, or at least 5α-DHP in maternal circulation, have biological relevance; that is, they may not be inactive. They could play some role in maintenance of equine pregnancy since inhibition of 3β-HSD, and therefore cessation of synthesis of progesterone, does not induce abortion in mares while it does induce abortion in other species.

Oregon Agriculture Experiment Station Technical Paper no. 10780. This work was supported by: USDA Competitive Animal Health Research Grant no. 053 and Oregon Agriculture Experiment Station Project no. 329. We would like to thank Covell West Meat Packing, Redmond, OR for allowing us to collect tissues, J. E. Bertrand for the critical reading of this manuscript and E. Bill for her assistance in the laboratory.

References

- Ainsworth L and Ryan KJ (1969) Steroid hormone transformations by endocrine organs from pregnant mammals. III. Biosynthesis and metabolism of progesterone by the mare placenta *in vitro* *Endocrinology* **84** 91–97
- Atkins DT, Harms PG, Sorensen AM and Fleeger JL (1976) Isolation, identification and quantitation of serum 5α-pregnane-3,20-dione and its relationship to progesterone in the pregnant mare *Steroids* **28** 867–880
- Creange JE, Anzalone AJ, Potts, GO and Schane HP (1981) Win 32,799, a new, potent intrauterine agent in rats and rhesus monkeys *Contraception* **24** 289–299
- Crooij MJ, deNooyer CCA, Rao BR, Berends GT, Gooren LJ and Janssens, J (1988) Termination of early pregnancy by the 3β-hydroxysteroid dehydrogenase inhibitor epistane *New England Journal of Medicine* **319** 813–817
- Dehennin L, Reiffsteck A and Scholer R (1980) Simple methods for the synthesis of twenty different, highly enriched deuterium labeled steroids, suitable as internal standards for isotope dilution mass spectrometry *Biomedical Mass Spectroscopy* **7** 493–499
- Fowden AL and Silver M (1987) Effects of inhibiting 3β-hydroxysteroid dehydrogenase on plasma progesterone and other steroids in the pregnant mare near term *Journal of Reproduction and Fertility Supplement* **35** 539–545
- Ginther OJ (1992) Embryology and Placentation. In *The Reproductive Biology of the Mare* (2nd Edn) pp 385–415 Ed. OJ Ginther. Equiservices, Wisconsin
- Hamon M, Clarke SW, Houghton E, Fowden AL, Silver M, Rossdale PD, Ousey JC and Heap RB (1991) Production of 5α-dihydroprogesterone during pregnancy in the mare *Journal of Reproduction and Fertility Supplement* **44** 529–535
- Holtan DW, Ginther OJ and Estergreen VL (1975) 5α-Pregnanes in pregnant mares *Journal of Animal Science* **41** 359–365
- Holtan DW, Squires EL, Lapin DR and Ginther OJ (1979) Effect of ovariectomy on pregnancy in mares *Journal of Reproduction and Fertility* **27** 457–463
- Holtan DW, Houghton E, Silver M, Fowden A, Ousey J and Rossdale PD (1991) Plasma progestagens in the mare, fetus and newborn foal *Journal of Reproduction and Fertility Supplement* **44** 517–528

- Houghton E, Dumasia MC, Teale P, Smith SJ, Cox J, Marshall D and Gower DB (1990) The use of stable isotopes and gas chromatography/mass spectrometry in the identification of steroid metabolites in the equine. *Steroids* **55** 433–439
- Knowles JE, Squires EL, Shideler RK, Tarr SF and Nett TM (1994) Progestins in mid to late pregnant mares *Journal of Equine Veterinary Science* **14** 34–38
- Ledger WL, Webster MA, Anderson ABM and Turnbull AC (1985) Effect of inhibition of prostaglandin synthesis on cervical softening and uterine activity during ovine parturition resulting from progesterone withdrawal induced by epostane *Journal of Endocrinology* **105** 227–233
- Martin PA, Hammitt DG, Strohbehn RS and Keister DM (1987) Induction of parturition in swine and epostane, a competitive inhibitor of 3β -hydroxysteroid dehydrogenase *Journal of Animal Science* **64** 497–506
- Moss GE, Estergreen VL, Becker SR and Grant BD (1979) The source of the 5α -pregnanes that occur during gestation in mares *Journal of Reproduction and Fertility Supplement* **27** 511–519
- Potts GO, Creanage JE, Harding HR and Schane HP (1978) Trilostane, an orally active inhibitor of steroid biosynthesis *Steroids* **32** 257–267
- Power SGA and Challis JRG (1987) Steroid production by dispersed cells from fetal membranes and intrauterine tissues of sheep *Journal of Reproduction and Fertility* **81** 65–76
- Schane HP, Pott GO and Creanage JE (1979) Inhibition of ovarian, placental and adrenal steroids in the rhesus monkey by trilostane *Fertility and Sterility* **32** 464–467
- Schutzer WE and Holtan DW (1996) Steroid transformations in pregnant mares: metabolism of exogenous progestins and unusual metabolic activity *in vivo* and *in vitro* *Steroids* **61** 94–99
- Silver M (1994) Placental progestagens in the sheep and horse and the changes leading to parturition *Experimental and Clinical Endocrinology* **102** 203–211
- Taylor MJ, Webb R, Mitchell MD and Robinson JS (1982) Effect of progesterone withdrawal in sheep during late pregnancy *Journal of Endocrinology* **82** 85–93