Concentrations of progesterone, 17 α -hydroxyprogesterone and 20 α -dihydroprogesterone in the plasma of mares during pregnancy and at parturition

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Summary. Plasma concentrations of progesterone and 17 α -hydroxyprogesterone were high in the 2nd and 3rd months of gestation, but 20 α -dihydroprogesterone increased from a level of 2 ng/ml, during the first 3 months, to 10–15 ng/ml during months 5–10, to reach 80–120 ng/ml during the last 30 days before foaling.

Introduction

In the mare the variations in plasma progesterone levels during pregnancy differ from those observed in other domestic animals. After a significant increase by Day 90 of gestation, progesterone values in the mare gradually decrease until approximately Day 210. Thereafter, the concentrations are relatively low up to the time of foaling (Bono, Brotto & Tamanini, 1974; Smith, 1974; Holtan, Nett & Estergreen, 1975b, c; Lovell, Stabenfeldt, Hughes & Evans, 1975).

Progesterone is known to be synthesized by the corpus luteum and the placenta. In the mare, these tissues also contain enzymes which are involved in the synthetic pathways to other progestagens, such as 17 α -hydroxyprogesterone, 20 α -dihydroprogesterone (Short, 1962; van Rensburg & van Niekerk, 1968; YoungLai, 1971; Flood & Marrable, 1975) and 20 α -hydroxy-5 α -pregnan-3-one (Holtan, Ginther & Estergreen, 1975a; Moss, Estergreen, Becker & Grant, 1979). While the changes in 17 α -hydroxyprogesterone and 20 α -hydroxy-5 α -pregnan-3-one concentrations throughout pregnancy in the mare have been studied (Holtan *et al.*, 1975b, c), nothing is known about the levels of 20 α -dihydroprogesterone present in peripheral plasma.

This study was therefore undertaken to characterize the patterns of this progestagen, 17 α -hydroxyprogesterone and progesterone in peripheral plasma of mares throughout pregnancy and parturition.

Materials and Methods

Blood samples were collected at 09:00 h from 98 randomly selected mares of standard breeding, 4–12 years of age. The mares were at various stages of pregnancy and the day of gestation was calculated from the dates of covering, i.e. mating. In addition, blood was taken daily from 4 mares for 15 days *pre partum* until 5 days *post partum*. Jugular vein blood was collected into heparinized tubes and was centrifuged immediately at 2300 g for 10 min. Plasma was removed and stored at –20°C until assay.

Progesterone, 17 α -hydroxyprogesterone and 20 α -dihydroprogesterone concentrations in each plasma sample were determined by the radioimmunoassay (RIA) procedure described by

Seren, Leopold & Bolelli (1974). A 0.5 ml aliquot of plasma was extracted with 5 ml ether. The ether was evaporated under a stream of nitrogen and the residue was chromatographed on 7.5×0.5 cm columns of Sephadex LH-20 using benzene:methanol (95:5 v/v) as eluant. Two fractions were then collected: one from 0.8 to 1.6 ml containing only progesterone and the other from 1.6 to 3.4 ml containing 17α -hydroxyprogesterone and 20α -dihydroprogesterone. Eluates were desiccated and the residue was redissolved in 1 ml ethanol. The fraction which contained only progesterone was subjected to assay with an antiserum raised in a rabbit to 11α -hydroxyprogesterone-hemisuccinate-BSA (1:15 000). The other fraction was divided in two aliquots which were used for assessment of the 17α - and 20α -progestagens by assays with antisera, raised in rabbits, to 17α -hydroxyprogesterone-3-oxime-BSA (1:27 000) and 20α -dihydroprogesterone-3-oxime-BSA (1:20 000), respectively.

Table 1. Cross-reactions (%) of various steroids with antisera raised against progesterone, 17α -hydroxyprogesterone and 20α -dihydroprogesterone

Steroids	Antiserum against		
	11α -Hydroxyprogesterone hemisuccinate-BSA	17α -Hydroxyprogesterone -3-oxime-BSA	20α -Dihydroprogesterone -3-oxime-BSA
Cholesterol	<0.001	<0.001	<0.001
<i>C21 Steroids</i>			
Pregn-4-ene-3,20-dione	100.0	3.0	<1.0
Pregn-4-ene- 17α -ol-3,20-dione	2.5	100.0	0.05
Pregn-4-ene- 20α -ol-3-one	0.5	0.05	100.0
Pregn-4-ene- 20β -ol-3-one	<1.0	<0.01	0.07
Pregn-4-ene- 11α -ol-3,20-dione	87.0	0.1	<0.01
Pregn-4-ene- 11β -ol-3,20-dione	16.0	0.01	<0.01
Pregn-4-ene- 14α -ol-3,20-dione	<2.0	0.2	<0.01
Pregn-4-ene- 16α -ol-3,20-dione	<2.0	0.04	0.07
Pregn-4-ene- 6α -ol-3,20-dione	<2.0	—	<0.01
Pregn-4-ene- 6β -ol-3,20-dione	<2.0	—	0.02
Pregn-4-ene- 11β , 17α , 21 -triol-3,20-dione	<0.001	<0.001	<0.01
Pregn-5-ene- 3β -ol-20-one	0.05	0.04	<0.01
5α -Pregnan-3,20-dione	<1.0	1.16	<0.01
20α -hydroxy- 5α -pregnan-3-one	0.01	<0.01	28.1
3β -hydroxy- 5α -pregnan-20-one	0.04	0.4	0.07
<i>C19 Steroids</i>			
Androst-4-ene- 17β -ol-3-one	<0.001	<0.01	<0.01
Androst-4-ene-3,17-dione	<0.001	<0.01	<0.01
Androst-5-ene- 3β -ol-17-one	<0.001	<0.01	—
<i>C18 Steroids</i>			
Oestra-1,3,5-triene-3,17 β -diol	<0.001	<0.001	<0.01
Oestra-1,3,5-triene-3-ol-17-one	<0.001	<0.001	<0.001

Table 2. Data pertaining to the radioimmunoassays for progesterone, 17α -hydroxyprogesterone and 20α -dihydroprogesterone

	Progesterone	17α -Hydroxyprogesterone	20α -Dihydroprogesterone
Sensitivity (pg/tube)	13 (10)	4 (10)	5 (10)
Coefficient of variation			
Intra-assay (%)	6.0 (10)	5.2 (10)	4.7 (10)
Inter-assay (%)	6.3 (10)	6.0 (10)	5.8 (10)
Blank (ng/ml)	<0.01 (10)	0.01 (10)	<0.02 (10)
Recovery (%) (mean $\pm$ s.e.m.)	83.5 $\pm$ 0.63 (30)	81.6 $\pm$ 0.75 (30)	78.5 $\pm$ 0.48 (190)

No. of observations is given in parentheses.

Text-fig. 1. Plasma concentrations of (a) progesterone, (b) 17 α -hydroxyprogesterone and (c) 20 α -dihydroprogesterone in 98 mares throughout pregnancy. The pattern of the hormone concentration is given by the continuous line which was obtained by means of a least square cubic spline approximation.

The cross-reactions of each antiserum to the three hormones studied and to other steroids are given in Table 1, and the assay performances are shown in Table 2.

Results

The hormone concentrations at various stages of pregnancy are shown in Text-fig. 1. Progesterone values increased significantly at about Day 90 of gestation followed by a gradual decrease at about Day 240. From Days 240 to 300 progesterone levels were particularly low (< 2 ng/ml). In the last month of pregnancy progesterone rose again to values of 3–4 ng/ml (see Text-fig. 1a).

Plasma 17 α -hydroxyprogesterone levels were < 1.5 ng/ml throughout gestation, except for a marked increase to 2–4 ng/ml between Days 40 and 70 (see Text-fig. 1b).

The concentration of 20 α -dihydroprogesterone was < 2 ng/ml up to Day 100 when it reached 10–15 ng/ml at Days 150–180. Concentrations then remained unchanged until about Day 300. Within the last 30 days of gestation, there was a dramatic increase to give peak values of 80–120 ng/ml around foaling (see Text-fig. 1c).

The hormone changes during parturition are illustrated in Text-fig. 2. In these mares the pattern and levels of the three progestagens were similar to those observed in the mares of the previous group at the same stage of gestation. Just before foaling, the concentrations of all the hormones fell, the drop in 20 α -dihydroprogesterone concentrations being particularly evident. After foaling the values of all 3 steroids remained low, < 1 ng/ml.

Text-fig. 2. Plasma concentrations of progesterone (●), 17 α -hydroxyprogesterone (■) and 20 α -dihydroprogesterone (▲) during late gestation and parturition in 4 mares. Values are mean $\pm$ s.e.m.

Discussion

The present findings on plasma progesterone levels in the mare during pregnancy and parturition are similar to those recorded by Bono *et al.* (1974), Smith (1974), Holtan *et al.* (1975b) and Lovell *et al.* (1975). But they are in contrast to those of Noden, Oxender & Hafs (1978), who found progesterone levels as high as 6–16 ng/ml throughout pregnancy. However, the antiserum used by Noden *et al.* (1978) was not highly specific; it cross-reacted with 5 α -pregnan-3,20-dione (50%) and 3 β -hydroxy-5 α -pregnane-20-one (40%), and the exact concentrations of these progestagens were not known.

The pattern of 17 α -hydroxyprogesterone changes was in agreement with that observed by Holtan *et al.* (1975c), who utilized a competitive protein binding assay. The increase of this hormone in the 2nd and 3rd month of pregnancy, and the increase in progesterone at the same time, coincides with high PMSG levels in the mare (Nett, Holtan & Estergreen, 1975; Noden *et al.*, 1978). At this stage of pregnancy, secondary corpora lutea become detectable, increasing steadily in number until Day 160 of gestation (Squires, Douglas, Steffenhagen & Ginther, 1974). These corpora lutea would synthesize progesterone and 17 α -hydroxyprogesterone.

Maximum concentrations of 20 α -dihydroprogesterone were found in the last month of pregnancy, but the source of this hormone is uncertain. The antiserum which we used to measure 20 α -dihydroprogesterone had some cross-reactivity against 20 α -hydroxy-5 α -pregnan-3-one (Table 1) and our values could therefore include a contribution by this 5 α -pregnane derivative. During the oestrous cycle Short (1962) found that luteal tissue of the mare contained, in addition to progesterone, large amounts of 20 α -dihydroprogesterone. Luteal cells would hence contain an active 20 α -reductase enzyme system. Similarly, according to van Rensburg & van Niekerk (1968) and YoungLai (1971), granulosa cells would produce 20 α -dihydroprogesterone after luteinization. This production, however, ceases during pregnancy (van Rensburg & van Niekerk, 1968). Flood & Marrable (1975), by means of a histochemical study of steroid metabolism in the equine fetus and placenta in the first 2 months of pregnancy, found a marked ability for synthesis of 20 α -dihydroprogesterone in placental and trophoblast tissues. Moss *et al.* (1979) found high levels of 20 α -hydroxy-5 α -pregnan-3-one in the uterine artery and vein of Pony mares between 272 and 294 days of gestation but they did not examine the peripheral plasma concentrations.

There is release of prostaglandin (PG) F-2 α in mares in late pregnancy (Silver *et al.*, 1979). In the rat, PGF-2 α is a potent inducer of 20 α -hydroxysteroid dehydrogenase (Hall & Robinson, 1978), and the changes of plasma 20 α -dihydroprogesterone recorded in this study may be related to the increased PGF-2 α .

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References

- Bono, G., Brotto, G. & Tamanini, C. (1974) La concentrazione plasmatica degli estrogeni e del progesterone nella cavalla durante la gravidanza. *Atti Soc. It. Sci. Vet.* **28**, 345–351.
- Flood, P.F. & Marrable, A.W. (1975) A histochemical study of steroid metabolism in the equine fetus and placenta. *J. Reprod. Fert., Suppl.* **23**, 569–573.
- Hall, A.K. & Robinson, J. (1978) Progesterone, 20 α -dihydroprogesterone and PGF; interrelated hormonal changes during pseudopregnancy in the rat. *Prostaglandins* **15**, 1013–1025.
- Holtan, D.W., Ginther, O.J. & Estergreen, V.L. (1975a) 5 α -pregnanes in pregnant mares. *J. Anim. Sci.* **41**, 359.
- Holtan, D.W., Nett, T.M. & Estergreen, V.L. (1975b) Plasma progestins in pregnant, postpartum and cyclic mares. *J. Anim. Sci.* **40**, 251–260.
- Holtan, D.W., Nett, T.M. & Estergreen, V.L. (1975c) Plasma progestagens in pregnant mares. *J. Reprod. Fert., Suppl.* **23**, 419–424.
- Lovell, J.D., Stabenfeldt, G.H., Hughes, J.P. & Evans, J.W. (1975) Endocrine pattern of the mare at term. *J. Reprod. Fert., Suppl.* **23**, 449–456.
- Moss, G.E., Estergreen, V.L., Becker, S.R. & Grant, B.D. (1979) The source of the 5 α -pregnanes that occur during gestation in mares. *J. Reprod. Fert., Suppl.* **27**, 511–519.
- Nett, T.M., Holtan, D.W. & Estergreen, V.L. (1975) Oestrogens, LH, PMSG and prolactin in serum of pregnant mares. *J. Reprod. Fert., Suppl.* **23**, 457–462.
- Noden, P.A., Oxender, W.D. & Hafs, H.D. (1978) Plasma luteinizing hormone, progestagens and estrogens in mares during gestation, parturition and first postpartum estrus (foal estrus). *Am. J. Vet. Res.* **39**, 1964–1972.
- Seren, E., Leopold, A. & Bolelli, G. (1974) Peripheral plasma levels of oestrogens and progesterone during the bovine oestrus cycle. *Arch. Vet. It.* **25**, 1–20.
- Short, R.V. (1962) Steroid in the follicular fluid and the corpus luteum of the mare. A "two-cell type" theory of ovarian steroid synthesis. *J. Endocr.* **50**, 589–597.
- Silver, M., Barnes, R.J., Comline, R.S., Fowdel, A.L., Clover, L. & Mitchell, M.D. (1979) Prostaglandins in maternal and fetal plasma and in allantoic fluid during the second half of gestation in the mare. *J. Reprod. Fert., Suppl.* **27**, 531–539.

- Smith, I.D.** (1974) Progesterone concentrations in the peripheral plasma of the mare during pregnancy. *Res. Vet. Sci.* **16**, 114–116.
- Squires, E.L., Douglas, R.H., Steffenhagen, W.P. & Ginther, O.P.** (1974) Ovarian changes during the estrous cycle and pregnancy in mares. *J. Anim. Sci.* **38**, 330–338.
- van Rensburg, S.J. & van Niekerk, C.H.** (1968) Ovarian function, follicular oestradiol-17 β and luteal progesterone and 20 α -hydroxy-pregn-4-en-3-one in cycling and pregnant equines. *Onderstepoort J. vet. Res.* **35**, 301–318.
- YoungLai, E.V.** (1971) Steroid content of the equine ovary during the reproductive cycle. *J. Endocr.* **50**, 589–597.

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