PLASMA PROGESTERONE LEVELS IN THE MARE DURING THE OESTROUS CYCLE AND PREGNANCY

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ABSTRACT: Terblanche H M; Maree L. Plasma progesterone levels in the mare during the oestrous cycle and pregnancy. Journal of the South African Veterinary Association (1981) 52 No. 3, 181–185 (En) Department of Genesiology, Faculty of Veterinary Science, University of Pretoria, P.O. Box 12580, 0110 Onderstepoort, Republic of South Africa.

Plasma progesterone was determined with the aid of a competitive protein-binding assay in mares during the oestrous cycle, early pregnancy (45–60 days) and later pregnancy (2–10 months). Progesterone levels were low during oestrus (< 1 ng per ml) (3,18 nmol/l) and reached high levels (often in excess of 10 ng per ml) (31,8 nmol/l) within 3–4 days after ovulation. The high luteal levels were maintained for approximately 5–8 days and then declined sharply over a period of approximately 24–48 hours to reach low levels at the subsequent oestrus period.

In mares conceiving after service, the progesterone levels rose rapidly to 5–9 ng per ml (15,9–28,6 nmol/l) 21 days after service. Levels of 4–10 ng per ml (12,7–31,8 nmol/l) were found between 30 and 60 days after successful service with a tendency towards lower levels from 30–42 days and higher levels from 42–60 days. Progesterone levels remained between 7 and 10 ng per ml (22,3–31,8 nmol/l) from 60–110 days and thereafter fell to a relatively constant level of 3–6 ng per ml (9,5–19,1 nmol/l) until the tenth month of pregnancy.

The blood samples were centrifuged within one hour of collection and the plasma transferred to 10 ml plastic bottles. The samples were stored at –15°C until assayed for progesterone content.

**INTRODUCTION**

The biochemical nature and physiological functions of progesterone are well known following the tremendous amount of research performed since its isolation in pure form in 1934 as reported by Grepf. It is known to be of importance in the oestrous cycle as well as in pregnancy and various studies have therefore been performed to elucidate the hormonal state of the non-pregnant and pregnant mare as far as progesterone is concerned.

These studies have resulted in a better understanding of the physiological processes during both the oestrous cycle and pregnancy and this knowledge will enable scientists and veterinarians to improve the reproductive efficiency of domestic animals including the horse. Due to the different methods used for the assay of progesterone levels, e.g. gas chromatography, radioimmunoassay (RIA), and competitive protein-binding assay (CPBA), and the variation in progesterone levels reported as a result thereof, individual researchers will have to interpret their results according to the assay used.

The present study was undertaken to establish the applicability of a recently developed rapid CPBA for bovine progesterone to the measurement of progesterone levels during the oestrous cycle and pregnancy of mares and to establish a range of normal values in the mare during the oestrous cycle and pregnancy.

**MATERIALS AND METHODS**

**Animals and samples**

Blood samples were collected in heparin at 08h00 at 2 day intervals from 2 mares (B2 and A2) during 3 consecutive oestrus cycles and from 2 mares (G11 and G2) from oestrus and service to early pregnancy on 45–60 days. Similar samples were collected from a mare (A3) between the 22nd and 52nd day of pregnancy; from one mare (B9) between the second and sixth month of pregnancy; from one mare (B8) between the fourth and eighth month of pregnancy and from one mare (A2) between the sixth and tenth month of pregnancy.

**RESULTS**

Progesterone levels determined during oestrus were generally very low (<0,5 ng per ml) (1,6 nmol/l) except in the case of 2 oestrus periods of mare A2 where the levels averaged approximately 1,0 ng per ml (3,2 nmol/l). Three to 4 days after ovulation progesterone reached high levels, often in excess of 10 ng per ml (31,8 nmol/l), where it remained for approximately 5–8 days. A rapid decline then occurred over a period of 24–48 hours to the previously described low levels during the subsequent oestrus (Fig. 1 and 2).

In mares conceiving the progesterone levels 21 days after service ranged between 5,0 and 9,0 ng per ml (15,9 and 28,6 nmol/l) with a slight drop thereafter to 3,0 to 7,0 ng per ml (9,5–22,3 nmol/l) until the 35th to 42nd day. Thereafter the progesterone levels increased again to 7,0–10,0 ng per ml (22,3–31,8 nmol/l) at approximately 60 days (Fig. 2 and 3).

The progesterone levels remained between 7,0 and 10,0 ng per ml (22,3–31,8 nmol/l) between days 60 and 110 and then declined slowly over a period of approximately 20 days to a relatively constant level of 3,0 to 6,0 ng per ml (9,5–19,1 nmol/l) until the tenth month of pregnancy (Fig. 4–6).

**DISCUSSION**

The rapid CPBA used with success for the determina-
Fig. 1 Progesterone levels in two mares during the oestrous cycle

Fig. 2 Progesterone levels in two mares from oestrus to early pregnancy

Fig. 3 Progesterone levels in a mare during early pregnancy
Fig. 4 Progesterone levels in a mare between the second and sixth month of pregnancy

Fig. 5 Progesterone levels in a mare between the fourth and eighth month of pregnancy

Fig. 6 Progesterone levels in a mare between the sixth and tenth month of pregnancy
tion of bovine progesterone\textsuperscript{19} was successfully applied to the determination of plasma progesterone in the mare during the oestrous cycle and pregnancy. This conclusion is based on the good correlation found between the present results and those reported by others as will be discussed below.

The time consuming column chromatographic step employed by Van Niekerk, Morgenthau, Sanders & Malan\textsuperscript{16} was omitted in the present assay without sacrifice in the reliability of the assay. The steroid of major importance in the mare as far as interference and competition with progesterone is concerned and therefore of importance in the reliability of the assay, is 17\alpha-hydroxy-progesterone\textsuperscript{20}. According to Short\textsuperscript{13} this steroid however occurs in low levels in the mare and this would tend to lessen its competitive effect which was reported by van Niekerk et al\textsuperscript{16}. In addition, Fassora and Luisi\textsuperscript{1} have reported on its limited displacement affinity for progesterone in the binding system employed and this, together with the relatively low recovery rate of the steroid after petroleum ether extraction\textsuperscript{15}, has led us to ignore its possible influence on the progesterone assay employed.

The progesterone levels reported in the present study during oestrus in the mare are in very good agreement with the results of many other workers\textsuperscript{5,6,10-11} but are not as low as those reported by Plotka, et al\textsuperscript{12} and Van Niekerk et al\textsuperscript{16}. The former authors, however, used the more sensitive RIA.

The peak luteal levels of 10 ng per ml (31,8 nmol/ml\textsuperscript{6}) reported in this study are somewhat higher than the levels reported by Allen & Hadley\textsuperscript{1}, Ganjam, Kenney & Flickinger\textsuperscript{6} and van Niekerk et al\textsuperscript{16} but are comparable to the mid-dioestrous average of 13,6 ng per ml (43,25 nmol/ml\textsuperscript{7}) reported by Noden, Oxender & Hafs\textsuperscript{11}. The levels in excess of 10 ng per ml (31,8 nmol/ml) reported in this study never exceeded 14 ng per ml (44,5 nmol/ml) when read from a log/logit plot\textsuperscript{15}.

Progesterone levels in early pregnancy reported in the present study are in good agreement with those reported by Benjaminse & Tomasaarda\textsuperscript{2} and Holtan, Nett & Estergreen\textsuperscript{9} but are slightly lower than those of Allen & Hadley\textsuperscript{1} and higher than those of van Niekerk et al\textsuperscript{16}. At 30 days after ovulation and service, present results correspond well with those of Allen & Hadley\textsuperscript{1} and Holtan, Nett & Estergreen\textsuperscript{9} but are again higher than those of van Niekerk et al\textsuperscript{16}.

The decrease in progesterone levels observed by Allen & Hadley\textsuperscript{1} at day 30, by Ganjam & Kenney\textsuperscript{3} and Ganjam, Kenney & Flickinger\textsuperscript{6} between day 9 and 21 weeks and by van Niekerk et al\textsuperscript{16} between days 37 and 42 were seen in the present study between days 25 and 42. The levels at this stage of 3-7 ng per ml (9,5-22,3 nmol/ml\textsuperscript{9}) are in good agreement with the 5 ng per ml (15,9 nmol/ml\textsuperscript{7}) reported by Allen & Hadley\textsuperscript{1} and Holtan, Nett & Estergreen\textsuperscript{9} but are slightly higher than the 1,5-3,5 ng per ml (4,8-11,1 nmol/ml\textsuperscript{7}) reported by van Niekerk et al\textsuperscript{16} between days 10 and 42.

The present results of 7-10 ng per ml (22,3-31,8 nmol/ml\textsuperscript{11}) measured between days 42 and 110 are comparable at the higher levels with the results of Allen & Hadley\textsuperscript{1} but are lower than those of Ganjam & Kenney\textsuperscript{3} and Ganjam, Kenney & Flickinger\textsuperscript{6} at 2 months as well as those reported by Holtan, Nett & Estergreen\textsuperscript{9}. Present results compare favourably with those of van Niekerk et al\textsuperscript{16} although their results only go as far as day 52.

The levels of 3-6 ng per ml (9,5-19,1 nmol/ml\textsuperscript{7}) recorded in the present study from approximately 4-10 months are in good agreement with the 4-5 ng per ml (12,7-15,9 nmol/ml\textsuperscript{7}) reported by Ganjam & Kenney\textsuperscript{3} and Ganjam, Kenney & Flickinger\textsuperscript{6} but are higher than the levels reported by Holtan, Nett & Estergreen\textsuperscript{9} and lower than the higher levels reported by Allen & Hadley\textsuperscript{1}. The very high levels of 12,5 to 20 ng per ml (39,8-63,6 nmol/ml\textsuperscript{7}) in the first 3 months of pregnancy and 25-60 ng per ml (79,5-190,8 nmol/ml\textsuperscript{7}) in the last 3 months of pregnancy reported by Burns & Fleeger\textsuperscript{2} were not found in the present study. These authors, however, used RIA for total progestagens compared to the present CPBA which is relatively specific for progesterone due to the use of petroleum ether for extraction of progesterone\textsuperscript{15}. The varying results of Nitschel & van der Horst\textsuperscript{14} using gas chromatography, could not be correlated with the results of the present study.

The decline in progesterone levels observed during early pregnancy in this study around the 42nd day is consistent with the results of van Niekerk et al\textsuperscript{16} and those of van Rensburg & van Niekerk\textsuperscript{15}. The increase observed after the 42nd day of pregnancy is again in accordance with the results of van Niekerk et al\textsuperscript{16} and is probably due to the ovulation of tertiary follicles and formation of tertiary corpora lutea at this time.

The decline in progesterone levels reported in this study after day 110 is in agreement with the results of Ganjam & Kenney\textsuperscript{3}, Ganjam, Kenney & Flickinger\textsuperscript{6} and Holtan, Nett & Estergreen\textsuperscript{9} and could be explained by the well-known transition from luteal to placental progesterone control of pregnancy at approximately this time, although there still appears to be considerable doubt concerning the actual mechanism involved\textsuperscript{3}.

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REFERENCES


