TOTAL UNCONJUGATED OESTROGEN AND PROGESTERONE CONCENTRATIONS IN PERIPHERAL BLOOD DURING PREGNANCY IN THE DOG

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(Received 9th November 1974)

Summary. Daily blood samples were withdrawn from three bitches throughout gestation and at intervals during parturition. The highest mean oestrogen value of 26.1 ± 6.0 pg/ml was detected on the last day of pro-oestrus, and a lower post-oestrous peak occurred shortly after the maximum progesterone value of 17.0 ± 1.0 ng/ml which was recorded 18 days after first service. No oestrogen rise was detected before parturition, and progesterone could not be detected after the end of parturition.

INTRODUCTION

Little information is available on the endocrinology of pregnancy in the bitch. At various intervals during gestation in this species, progesterone concentrations have been determined in peripheral plasma by Parkes, Bell & Christie (1972) and Spano, Tietz, Masken & Hopwood (1971), and in serum by Smith & McDonald (1974).

Johnson, Davis & Hotchkiss (1972) studied changing plasma oestrogen concentrations in relation to sodium retention during canine pregnancy, and Metzler, Eletheriou & Fox (1966) reported variations in unconjugated oestrogen levels at weekly intervals in the gestation period of five bitches. Jones & co-authors (1973) simultaneously determined progesterone and oestradiol-17β levels during four canine pregnancies, but the frequency of sampling was not indicated.

In the current study, total unconjugated oestrogen and progesterone concentrations were determined for peripheral blood samples obtained daily during gestation and at frequent intervals immediately before and during parturition.

MATERIALS AND METHODS

One beagle and two mongrel nulliparous bitches were used in this study. The animals were examined daily for signs of vulval swelling or bleeding, and they were ‘tried’ daily with a male dog from the commencement of pro-oestrous bleeding until the end of oestrus. The bitches, T, B and C, were served on nine, six and five occasions, respectively. Vaginal smears were taken daily from each bitch from the beginning of pro-oestrus until 2 to 4 days after the end of
acceptance. Pregnancy was confirmed by abdominal palpation during the 4th week of gestation.

Blood samples (5 ml) were drawn daily from either the cephalic or saphenous vein of each bitch from the onset of pro-oestrus until 7 days after parturition. Samples were also taken at intervals immediately before and during parturition. In all cases, the blood sample was drawn into a syringe containing 500 i.u. heparin and was then immediately transferred to plastic centrifuge tubes in which the samples were frozen and stored at −20°C until they were assayed.

Total unconjugated oestrogen and progesterone concentrations were measured by the method of Challis, Heap & Illingworth (1971), as described by Hadley (1973, 1975). The oestrogen assay gave a recovery of 77·5% and a blank value of 9 pg/ml, and in the progesterone assay a recovery of 89% and a blank value of 0·3 ng/ml was obtained. The results were corrected for recovery and blank values in each case.

RESULTS

The total unconjugated oestrogen concentrations during pregnancy are shown in Text-fig. 1. The mean length of pro-oestrus and the interval from first acceptance to first service were calculated, and the daily values for each of these phases were transformed to the appropriate day in the 'average' period on a %
Oestrogen and progesterone in canine pregnancy

Text-fig. 2. Total unconjugated oestrogen and progesterone concentrations at parturition of Bitches B (○), C (●) and T (▲).

basis, i.e. if the duration of pro-oestrus in one bitch was 5 days, then the value for Day 1 was recorded at a time corresponding to 20% of the mean duration of pro-oestrus for the group. The mean values for each day of the uniform pro-oestrous and pre-service phases were then calculated. From first service, the mean daily values were calculated directly. Bitch T produced six pups, B nine and C ten, with one pup born dead in the litters from B and C. Each bitch whelped 63 days after first service.

On the first day of pro-oestrus, the total unconjugated oestrogen levels in the three bitches ranged from 9·2 to 15·7 pg/ml. Oestrogen concentrations then rose steadily to give the peak values of 21·9 pg/ml on the penultimate day of pro-oestrus in one animal (T) and 30·9 pg/ml and 39·7 pg/ml on the last day of this phase in the other two (B and C). A reduction to less than one-third of these levels occurred over the following 3 to 5 days in each case. Bitches C and T were first served 3 days after the oestrogen peak and B was served on the 4th day.

Oestrogen concentrations remained low during the last part of oestrus with a mean±S.E.M. value of 4·4±1·8 pg/ml on the last day of oestrus. These low levels were maintained for a further 4 days and then the mean daily concentrations rose to their highest post-oestrous values 23 and 25 days after first service. The first of these 2 days gave the highest post-oestrous value during gestation which was 10·2±1·7 pg/ml. The mean of the individual peak values during the three pregnancies was 14·1±4·4 pg/ml, and these occurred on Days 20, 23 and 25 of gestation in C, B and T, respectively. After the post-oestrous peak, oestrogen levels fell quickly and the mean concentrations were usually between 2 and 6 pg/ml for the rest of gestation. No pre-partum oestrogen rise
Text-fig. 3. A comparison between the total unconjugated oestrogen concentrations during (a) an oestrous cycle and (b) a succeeding pregnancy of one bitch. P-O, pro-oestrus; O, oestrus; M, day of first mating; W, day of whelping.

was recorded in any of the pregnancies studies (Text-fig. 2), and levels were undetectable in Bitch T throughout the last 10 days of gestation.

The oestrogen values recorded in Bitch B during an oestrous cycle and the succeeding pregnancy are compared in Text-fig. 3.

The pattern of progesterone levels during gestation are shown in Text-fig. 1. On the 1st day of pro-oestrus, a mean value of $0.2 \pm 0.1$ ng/ml was obtained. Levels remained low during this phase, but began to increase 3 days before the end of pro-oestrus to give a value of $1.2 \pm 0.4$ ng/ml on Day 1 of oestrus. The increase in progesterone levels continued slowly during the first 3 days of
oestrus, and then rapidly during the following 5 days before becoming more gradual from the last 2 days of oestrus. On the last day of oestrus, the mean value was $11.3 \pm 2.8$ ng/ml.

Progesterone concentrations continued to rise steadily until the highest mean daily value of $17.0 \pm 1.0$ ng/ml was reached 18 days after first service. The mean of the highest individual values of each pregnancy was $18.0 \pm 1.6$ ng/ml and these occurred 8, 8 and 10 days after the end of oestrus in the three pregnancies. The mean daily values declined from the peak level for 7 days, after which they fluctuated between 6 and 8 ng/ml for 13 days before falling at a progressively slower rate to $1.1 \pm 0.3$ ng/ml on the day before whelping. Levels continued to fall in the 24 hr before parturition (Text-fig. 2), and were undetectable 30 min after the last fetus of each litter had been delivered.

The progesterone concentrations recorded in Bitch B during an oestrous cycle and the succeeding pregnancy are compared in Text-fig. 4.

Text-fig. 4. A comparison between the progesterone concentrations during (a) an oestrous cycle and (b) a succeeding pregnancy in one bitch. P-O, pro-oestrus; O, oestrus; M, day of first mating; W, day of whelping.
DISCUSSION

The changes in the total unconjugated oestrogen concentrations in the peripheral blood of mated bitches were similar to those previously reported in unmated animals during oestrus (Hadley, 1975), with the mean peak oestrogen level occurring on the last day of pro-oestrus in the bitches which were subsequently served. This was 1 day earlier than in the non-pregnant bitches and 3 days earlier than Torbit, Abel, Tietz & Barran (1971) observed in bitches which had been served.

The oestrogen level rose shortly after oestrus, as reported in non-pregnant bitches (Hadley, 1975), and this was clearly marked between 7 and 16 days after oestrus (i.e. between 16 and 25 days after first service). This corresponds well with the observation of Metzler et al. (1966) that the highest level of total unconjugated oestrogens in the peripheral plasma of the bitch occurs at 3 to 4 weeks of gestation. Johnson et al. (1972), however, recorded the highest post-oestrous oestrogen concentrations in their study at the later stage of 25 to 42 days of gestation. The rise in oestrogen levels in the blood seen in the present study began when the progesterone concentrations were high and were maintained for 7 days after the progesterone levels started to decline before they were reduced for the remainder of gestation. It is probable that the CL is the source of the oestrogen production in gestation because the pattern of oestrogen changes is similar to that of the non-pregnant animal. The oestrogen concentrations during gestation were slightly lower than those in the normal cycle. Jones et al. (1973) also reported a post-oestrous rise of oestadiol-17β in the peripheral plasma of the pregnant bitch, but they considered the concentrations of this hormone to be slightly higher in gestation than in the non-pregnant animal during metoestrus.

No rise in oestrogen before parturition could be demonstrated in this study, although one bitch gave a single raised value of 15.5 pg/ml on the 3rd day before whelping. This agrees with the findings of Jones et al. (1973) and Metzler et al. (1966), but the oestrogen rise after parturition which the latter authors reported was not observed. This finding is in marked contrast to the clear pre-partum rise in plasma oestrogen seen in the ewe (Challis, 1971) and the cow (Henricks, Dickey, Hill & Johnston, 1972).

The mean daily progesterone values were similar during gestation to those reported earlier during the oestrous cycle (Hadley, 1975). In each of the three pregnancies studied, the peak progesterone level was slightly less than that of the preceding normal cycle. Parkes et al. (1972) reported similar values during the oestrous cycle and pregnancy in the bitch, as did Spano et al. (1971) and Sokolowski (1973). The similarity of progesterone concentrations in these two differing physiological states may reflect the fact that the CL is required throughout pregnancy (Marshall & Jolly, 1906; Sokolowski, 1971), and that the dog placenta contains little progesterone (Telegdy, Endröczi & Lissák, 1963). The highest mean progesterone concentrations were achieved 10 days after oestrus in the pregnant bitches. No evidence was seen of a post-oestrous fall in progesterone concentration followed by a large rise to peak gestational levels 19 to 24 days after the first day of oestrus, as described by Smith & McDonald (1972,
1974). Levels subsequently declined to approximately 1 ng/ml on the day before whelping. This decline in concentration was interrupted by a period of relatively constant values between 26 and 38 days after oestrus. It is possible that this may have resulted from a further LH release as described by Mattingley & co-authors (1970), although their observations were not supported by the later studies of Jones et al. (1973) and Smith & McDonald (1974).

The progesterone levels at whelping were below those of the non-pregnant bitch at the corresponding time after oestrus, as was reported by Smith & McDonald (1972), and progesterone was not detectable 30 min after the birth of the last fetus. This contrasts with the results of Parkes et al. (1972) and Spano et al. (1971), both of whom reported that values in pregnant bitches at whelping were similar to those of the non-pregnant bitch at the corresponding time after oestrus. The lower progesterone concentrations seen in the whelping and post-partum bitch are consistent with the shorter duration of pregnancy (63 days) compared to metoestrus (75 to 80 days) in the normal cycle.

ACKNOWLEDGMENTS

The author would like to thank Professor G. H. Arthur for his encouragement and for providing research facilities, and Dr D. E. Noakes for his criticism of the text. Technical assistance was given by Miss M. A. Lemon, Mrs J. D. Sutton and Mrs G. E. Clarke. Financial support was kindly provided by Glaxo Laboratories Limited, and Mrs M. Hughes typed the manuscript.

REFERENCES


