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Serum oestrogen, progesterone and prolactin concentrations in cyclic, pregnant and lactating beagle dogs

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Summary. No correlations between the three hormones measured were found in any of the reproductive states examined. In 2 out of 8 dogs a negative correlation between serum progesterone and prolactin levels was found at Day 11 and Day 15, respectively (Day 0 = first day of copulation). In all of the 8 animals in which the progesterone and prolactin concentrations were measured in the same samples in late pregnancy, a negatively correlated overall pattern was obtained between both hormone profiles. No correlation between serum oestrogen and prolactin levels was found.

It is concluded that the proliferative mammary gland changes which are induced in female dogs during long-term toxicity testing with progestagens are unlikely to be related to prolactin or oestrogen synthesis and/or secretion.

Introduction

Several recent studies of the concentrations of oestrogens, progesterone and LH during the oestrous cycle and pregnancy of the dog (Concannon, Hansel & Visek, 1975; Nett *et al.*, 1975; Smith & McDonald, 1974; Masken, 1972) have added considerably to our understanding of canine reproductive physiology. Such understanding is important because animal testing requirements for hormonal contraceptives and for progestagens and oestrogens used over long periods for non-contraceptive purposes include long-term toxicity studies in the dog. Certain progestagens used for human contraception have led to mammary gland changes in the dog, including enhanced occurrence of mammary gland neoplasia (Goldzieher & Kraemer, 1972; Wazeter, Geil, Berliner & Lamar, 1973; Hill & Dumas, 1974). The fact that the changes in the mammary gland have been found almost exclusively in the dog, rather than in rats, monkeys and man, has led to the assumption that the progestagens concerned are progestationally very active in the dog (Hill, Averkin, Brown, Gagne & Segre, 1970; Gräf, El Etreby, Richter, Günzel & Neumann, 1975; Haase, Beier, Hartmann & Elger, 1977), or that the canine mammary and/or anterior pituitary gland is very sensitive to progestagens (Neumann & Elger, 1972). A predominant role of progestagens in the stimulation of prolactin and growth hormone synthesis and/or secretion has therefore been suggested (Neumann & Elger, 1972). Only one preliminary report on radioimmunoassayable canine prolactin has been published (Jones, Brownstone & Boyns, 1976). A specific homologous radioimmunoassay for canine prolactin has now been developed (Gräf, Friedreich, Matthes & Hasan, 1977) and we have therefore investigated the relationship between prolactin, progesterone and oestrogen concentrations in the beagle dog during various reproductive states.

Materials and Methods

The beagle dogs were 2–5 years old, bred by Schering AG and maintained in indoor cages with access to an outdoor run during the day. They were fed a commercial diet (Altromin H) and provided with water *ad libitum*. Towards the anticipated start of pro-oestrus the animals were examined once or twice daily for signs of vulval swelling and for vaginal discharge. The first day of the appearance of sanguineous discharge from the vagina was regarded as the first day of pro-oestrus. For study of the cycle, blood sampling of 3 bitches was started 3–5 months after whelping and continued weekly until the start of the next pro-oestrus. Blood samples were then taken twice per week until the start of oestrus and thereafter weekly again for another 6–8 weeks. Mating was not allowed. All samples were related to the first day of pro-oestrus (Day 0).

In the pregnancy study all blood samples were related to the first day of tolerated copulation and were taken at different intervals once or twice per week. Three animals were killed a few days before parturition (Group 1); 3 were killed 4–7 days after parturition almost 16 h after removal of the pups (Group 2); 3 were killed 4–7 days after parturition but 1 h after suckling was allowed (Group 3); and 3 bitches were killed about 9 weeks after parturition, when lactation had terminated, i.e. 1–3 weeks after removal of the pups (Group 4). The mean duration of pregnancy for the 9 dogs in Groups 2, 3 and 4 was 63 $\pm$ 1 (s.e.m.) days.

The blood was collected by venepuncture between 07:00 and 10:00 h (regardless of the time of suckling), allowed to clot for about 4 h at 4°C and centrifuged at 3000 *g* for 10 min. The serum was removed and stored at −20°C until assayed.

In the 3 cyclic dogs progesterone, oestrogen and prolactin concentrations were measured. In the pregnancy and lactation study prolactin concentrations were determined in all 12 dogs, but because of insufficient quantities of serum progesterone levels were measured in only 8 animals (the 6 in Groups 1 and 3 and 2 in Group 4) and oestrogens only in the 3 dogs in Group 1.

Progesterone and oestrogen concentrations were estimated by radioimmunoassay according to the method previously described (Abraham, 1969; Hotchkiss, Atkinson & Knobil, 1971) but with minor modifications. Anti-progesterone serum was produced in this laboratory by immunizing sheep with progesterone-6α-succinate linked to purified bovine serum albumin, and the anti-oestradiol-17β serum was kindly provided by Dr B. Caldwell (Tillson, Thorneycroft, Abraham, Scaramuzzi & Caldwell, 1970). Serum prolactin concentrations were determined by the radioimmunoassay described previously with an antiserum raised in rabbits (Gräf *et al.*, 1977). Each serum sample was measured in duplicate. The concentrations of prolactin are expressed in terms of the canine prolactin reference preparation CPA 3 F4, which has a biological potency of about 24 i.u./mg when compared with the ovine reference preparation NIH-PRL-S12 (Gräf *et al.*, 1977). The sensitivities of the assays (per 0·8 ml incubation volume) were 6 pg progesterone, 2 pg oestradiol and 0·25 ng prolactin, and the mean overall intra-assay coefficients of variation were 4·3, 8·4 and 11.9% respectively.

Results

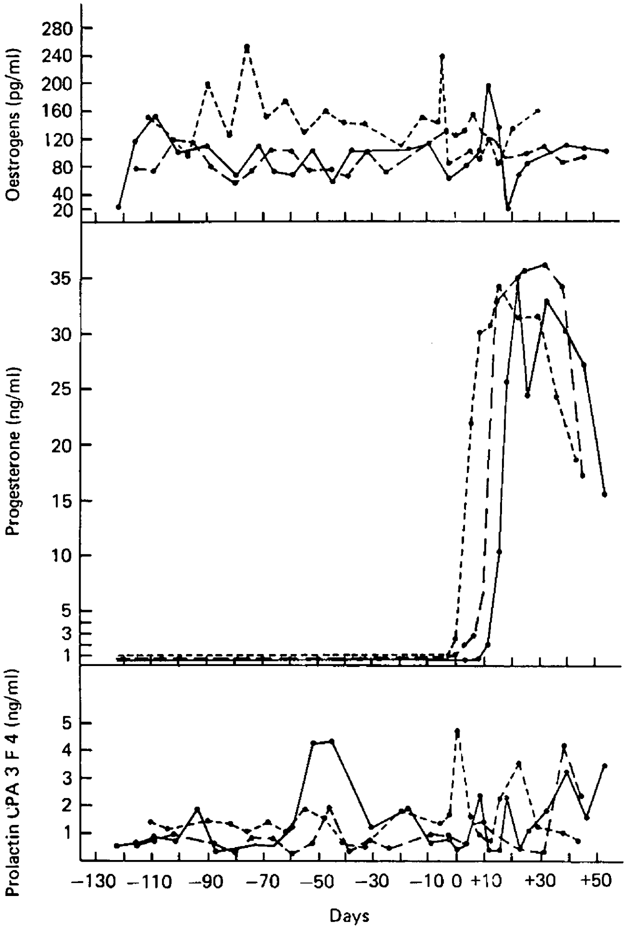
Oestrous cycle

The individual oestrogen, progesterone and prolactin profiles during the oestrous cycles of the 3 beagle dogs are shown in Text-fig. 1. Oestrogen concentrations did not show any distinct changes which could be related to particular stages of the cycle. However, a marked increase in progesterone level was found during late pro-oestrus and oestrus. During early metoestrus the progesterone concentrations reached maximal levels which remained high for about 20 days before declining slowly. The serum prolactin levels revealed no characteristic changes which could be related to the phases of the oestrous cycle.

Pregnancy

As in the cyclic animals, the oestrogen concentrations, in the 3 bitches in Group 1, during pregnancy showed marked individual fluctuations. Around copulation, the serum oestrogen concentrations ranged from 80 to 120 pg/ml; there was then a slight decline until mid-pregnancy (see Text-fig. 2c).

Serum progesterone concentrations (Text-fig. 2a) increased sharply during late pro-oestrus and oestrus and after copulation increased to maximum levels of about 50–70 ng/ml 2 weeks after copulation. The progesterone concentrations remained high until Day 35 and then decreased gradually until parturition. Examination of the progesterone concentrations in individual dogs (see Text-fig. 3) showed that, in 2 of the 8 animals investigated, at Days 11 and 15 after the first day of copulation, there was a sharp decrease in serum progesterone concentrations followed by a quick increase shortly



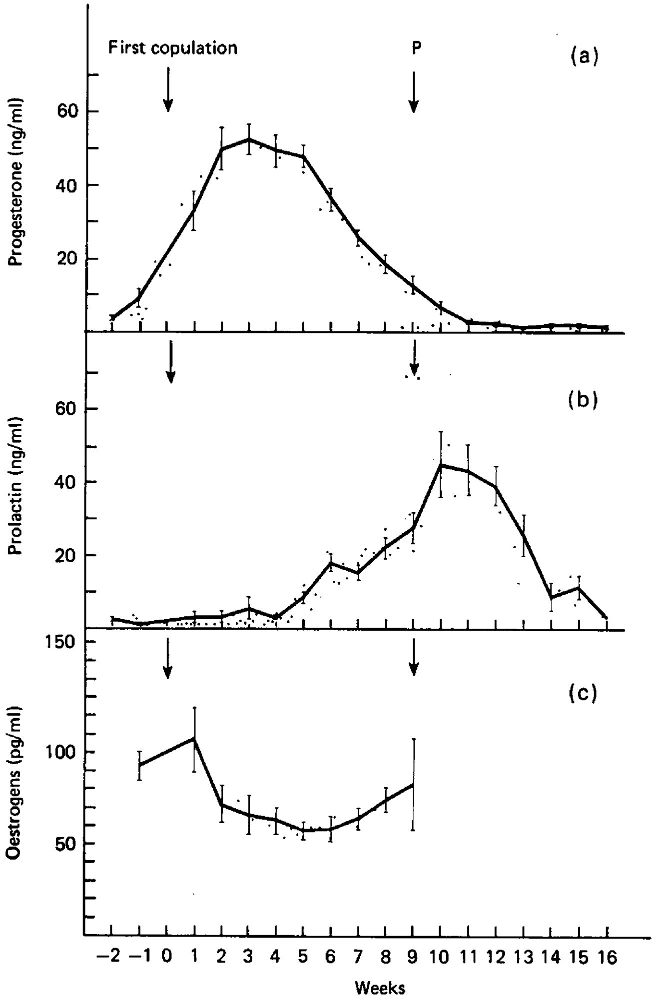
Text-fig. 1. Individual profiles of oestrogen, progesterone and prolactin concentrations in the serum of 3 beagle bitches throughout the oestrous cycle. Day 0 = first day of pro-oestrus. —— No. 1177; – – – No. 1112; ---- No. 594.

thereafter to give a second broader peak at about the same maximal level. Throughout lactation the serum progesterone concentrations remained basal (see Text-figs 2a and 3).

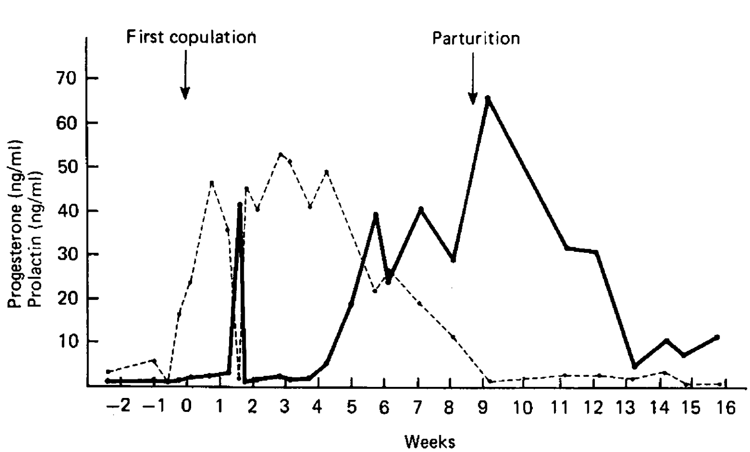
Serum prolactin concentrations remained within the basal range of about 1–5 ng/ml during the first half of pregnancy, except for some high values between Days 7 and 15. During the last half of pregnancy the prolactin concentrations increased constantly until parturition (see Text-figs 2b and 3). The high values between Days 7 and 15 of pregnancy were due to a dramatic increase in the prolactin concentrations (see Text-fig. 3) in 3 out of the 12 bitches studied. These prolactin surges coincided with the dramatic fall in serum progesterone concentrations which occurred at the same time in 2 animals. The failure to observe this negative correlation in the other 6 animals is almost certainly due to the timing of blood sampling, because the prolactin increase and the complementary progesterone decrease lasted only for a short while (probably only on 1 or 2 days), whereas blood samples were taken only once or twice per week.

Discussion

The profiles of oestrogen and progesterone found during the oestrous cycle and pregnancy in the present study are, in general, similar to those previously described by other investigators. Differences



Text-fig. 2. Serum concentrations of (a) progesterone in 8 bitches, (b) prolactin in 12 bitches and (c) oestrogens in 3 bitches throughout pregnancy and lactation. Day 0 = the first day of copulation. The vertical bars represent the mean ± s.e.m. of all samples measured in the preceding week. The dots are the mean values for each day if 2 or more samples were available. P = parturition.



Text-fig. 3. Serum progesterone (----) and prolactin (——) profiles of one beagle bitch throughout pregnancy and lactation (Group 4) (for details see 'Materials and Methods').

with respect to the absolute concentrations reported, especially for oestrogen, are probably due to the difference in the specificity of the antibodies used in the radioimmunoassays, and to the variable sampling intervals.

The oestrous cycle in the dog is characterized principally by increasing peripheral blood oestrogen levels during pro-oestrus, followed by a surge in LH concentrations in late pro-oestrus when serum progesterone concentrations start to increase to reach maximum levels in early metoestrus (Concannon *et al.*, 1975; Nett *et al.*, 1975; Hadley, 1975a; Jones *et al.*, 1973b; Masken, 1972); the increased oestrogen and LH concentrations decline within 1–4 days of ovulation to basal levels for the rest of the cycle. During pregnancy the progesterone concentrations remain maximal up to about Day 35 before waning steadily to give basal levels by the time of parturition or during the early stages of lactation (Austad, Lunde & Sjaastad, 1976; Concannon *et al.*, 1975; Hadley, 1975b; Smith & McDonald, 1974; Jones, Boyns, Bell, Christie & Parkes, 1973c; Masken, 1972; Christie, Bell, Horth & Palmer, 1971; Bell, Christie & YoungLai, 1971).

Jones *et al.* (1976) found increased plasma prolactin levels on the last day of pro-oestrus, but we were unable to find any clear changes in serum prolactin concentrations with the stage of the cycle. This was probably due to the times of blood sampling which were not frequent enough to detect short-term hormonal changes around ovulation. In this respect further more detailed studies are required to characterize this phase accurately.

In agreement with the results of Hadley (1975a, b) and Masken (1972), but unlike those of Nett *et al.* (1975), Concannon *et al.* (1975) and Jones *et al.* (1973c), I found similar oestrogen levels in pregnancy and during the cycle. In detail, the results presented suggest that between the 2nd and the 7th week of pregnancy lower oestrogen concentrations are to be found than during most of the cycle phases. Like Smith & McDonald (1974), Jones *et al.* (1973a, b, c) and Masken (1972), I found serum progesterone concentrations to be higher during early pregnancy than at metoestrus, although Concannon *et al.* (1975), Hadley (1975a, b) and Parkes, Bell & Christie (1972) reported fairly similar progesterone levels in pregnant and non-pregnant bitches. The dramatic decrease in progesterone levels at Days 11 and 15 after the first day of copulation, followed by a subsequent increase to maximum levels, was also reported by Smith & McDonald (1974). The second increase in serum progesterone concentrations, which almost coincides with the time of implantation, could result from an extra-ovarian source. However, the placenta contains only very small amounts of progesterone (Spano, Tietz, Masken & Hopwood, 1971) and there is also no evidence indicating any increase in adrenal progesterone production during pregnancy.

As found by Jones *et al.* (1976) and Gräf *et al.* (1977) serum prolactin concentrations increase steadily during the last half of pregnancy and reach high concentrations during early lactation. The present study supplements these findings and shows that during the later stages of lactation, from about Day 84 onwards (related to the first day of copulation), prolactin levels waned steadily until cessation of lactation. Furthermore there was a characteristic prolactin surge between Days 7 and 15 *post coitum*, which coincided with the sharp decrease in serum progesterone levels. These findings, together with the corresponding progesterone/prolactin profiles during late pregnancy and lactation (Gräf *et al.*, 1977), suggest that a negative correlation exists between these hormones and that the prolactin increase might mediate an inhibition of progesterone secretion, thereby implying that prolactin plays a key role within the mechanisms maintaining and terminating pregnancy. It seems unlikely, therefore, that administration of synthetic progestagens to the bitch would cause proliferative mammary gland changes indirectly via stimulated prolactin secretion. The absence of any positive correlation between serum oestrogen and prolactin levels suggests that oestrogens are probably not predominantly involved in the mechanisms responsible for the progestagen-induced proliferative mammary gland changes.

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