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PLASMA LEVELS OF ANDROSTENEDIONE, EPITESTOSTERONE, TESTOSTERONE AND OESTROGENS IN COWS AT PARTURITION

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SUMMARY

The concentration of total unconjugated oestrogens in the peripheral plasma of cows increased from 2·00 ± 0·20 (S.E.M.) ng/ml 7 days before to 3·87 ± 0·50 ng/ml 1 day before parturition and declined to values below 0·30 ng/ml on day 2 *post partum*. The concentrations of androstenedione, epitestosterone and testosterone remained approximately constant at 0·90 ± 0·10, 0·92 ± 0·08 and 0·40 ± 0·20 ng/ml respectively during the last week of pregnancy. After parturition the concentration of the three androgens declined rapidly.

Similar to data obtained in goats we did not find a marked increase in androgen levels in the peripheral blood of cows before parturition.

INTRODUCTION

Oestrogens in the peripheral plasma of the cow increase markedly during the last weeks of pregnancy, with peak values around parturition (Smith, Edgerton, Hafs & Convey, 1973; Dobson & Dean, 1974; Hoffmann, Wagner & Gimenez, 1976; Hoffmann, 1977; Stellflug, Han, Randel & Moody, 1978). Most of the oestrogens in cows appear to be derived from the placenta, production by the fetus being insignificant (Hoffmann, Wagner, Hixon & Bahr, 1979). Ainsworth & Ryan (1966) incubated bovine placental tissue with radioactive progesterone and pregnenolone, but they did not find any conversion into oestrogens. The origin of the oestrogen precursors during these last stages of pregnancy is not known, although bovine fetal placental tissue taken at term was able to convert both androstenedione and testosterone into oestrogens *in vitro* (Pierrepoint, Anderson, Griffiths & Turnbull, 1969).

We have therefore investigated whether there is any correlation between the concentration of androgens and the total unconjugated oestrogens in the peripheral blood of the cow from 1 week before until 1 week after parturition.

MATERIALS AND METHODS

Blood samples were taken daily by jugular venepuncture from eight pregnant cows from 1 week before until 1 week after parturition. Heparinized blood was centrifuged immediately (15 min, 600 *g*) and the plasma stored at −24 °C until used for the determination of testosterone and of total unconjugated oestrogens. For the determination of androstenedione and epitestosterone 12 ml blood samples were collected directly into tubes containing 2 ml methanol which inhibits in-vitro conversion of androstenedione to

epitestosterone (Bamberg, Choi & Möstl, 1978; Möstl, Choi & Bamberg, 1980). After centrifugation as above the supernatant fraction was stored at −24 °C.

The steroids were measured by radioimmunoassay (RIA). The antisera for androstenedione and testosterone were raised in rabbits in our laboratory (Nechansky, 1979) by the method of Vaitukaitis, Robbins, Nieschlag & Ross (1971) against 4-androsten-11α-ol-3,17-dione–hemisuccinate–bovine serum albumin and 4-androstene-11α,17β-diol-3-one–hemisuccinate–bovine serum albumin respectively. The antiserum for epitestosterone was purchased from Radioimmunoassay Ltd, Cardiff and that for total unconjugated oestrogens was kindly donated by Dr B. Cook, The Royal Infirmary, Glasgow. The steroid standards were purchased from Steraloids, Wilton, New Hampshire, U.S.A.

The RIA for androstenedione was carried out as follows. The steroid was extracted from a sample (0·5 ml) with 7·5 ml petroleum ether (40–60 °C b.p.), after addition of 100 µl 1 M-NaOH and [^{3}H]androstenedione (2000 disintegrations/min; NET-469, New England Nuclear, Dreieich, West Germany) in 0·05 ml of a solution of 0·1 M-phosphate buffer, pH 7·0, containing 9·0 g sodium chloride, 1·0 g sodium azide and 1·0 g gelatine per litre solution (GPBS). After extraction for 20 min on a mechanical shaker the aqueous phase was frozen during centrifugation at −20 °C, after which the organic phase was transferred to another tube and dried down under a stream of nitrogen. The residue was then redissolved in 0·25 ml of a mixture of benzene : methanol (95 : 5, v/v). After elution on a Sephadex LH-20 column (33 × 0·4 cm) with the same solvent mixture the fraction of 4–5 ml which corresponded to androstenedione was collected, dried down and redissolved in 0·4 ml GPBS.

For the RIA for androstenedione, 0·1 ml of the sample and standards were incubated overnight at 4 °C together with 0·1 ml antiserum (final dilution 1 : 5000, v/v) and 0·1 ml [^{3}H]androstenedione (20 000 disintegrations/min).

Free and bound steroids were separated by dextran-coated charcoal (0·5 ml of a suspension of 0·6% (w/v) charcoal and 0·06% (w/v) dextran T-70 in GPBS). The radioactivity of the antibody-bound steroid was measured in a liquid scintillation spectrometer and the amount of hormone in the sample estimated from a standard curve. After correcting for recoveries the results have been expressed as ng per ml sample (means ± S.E.M.; $n = 8$).

The RIA for epitestosterone was carried out in a manner similar to that for androstenedione with the following modifications: 0·5 ml samples were extracted with 5 ml diethyl ether after addition of [^{3}H]epitestosterone (2000 disintegrations/min; NET-252, New England Nuclear). After column chromatography on Sephadex LH-20 the eluate (fraction of 7–8 ml) was dried down and redissolved in 2 ml GPBS. In the RIA, 0·5 ml was incubated with 0·1 ml antibody dilution (1 : 4500, v/v) for 30 min at 37 °C and after addition of [^{3}H]epitestosterone (20 000 disintegrations/min) overnight in 0·1 ml GPBS at 4 °C.

The RIA for testosterone and that for total unconjugated oestrogens was performed according to the method of Nieschlag & Loriaux (1972), with the exception that thin-layer chromatography was not carried out. We found no differences in the concentrations of testosterone and total unconjugated oestrogens in peripheral plasma of cows whether chromatography was performed or not.

The final dilution of antibody was 1 : 6000 for the testosterone and 1 : 32 000 for the oestrogen RIA. The average recoveries after extraction ($n = 20$) were 65 ± 3·2 (S.E.M.)% for androstenedione, 71 ± 2·8% for epitestosterone, 84 ± 2·6% for testosterone and 77 ± 1·1% for oestrogens. The cross-reactions as calculated at 50% inhibition of binding were tested with the following steroids: cortisol, 17α-hydroxyprogesterone, progesterone, dehydroepiandrosterone, androstenedione, testosterone, 4-androsten-17α-ol-3-one, 5α-dihydrotestosterone, androst-5-ene-3β,17β-diol, androst-5-ene-3β,17β-diol, oestrone, oestradiol and oestriol. The antiserum for androstenedione showed cross-reactions with testosterone (2·4%), epitestosterone (6·6%) and 5α-dihydrotestosterone (0·4%); that for epitestosterone with androstenedione (3·7%), testosterone (1·8%) and 5α-dihydrotestosterone (0·4%); that

for testosterone with 5α-dihydrotestosterone (78%) and epitestosterone (0·6%); that for oestrogens with oestradiol-17β (110%), oestrone (100%) and oestriol (64%). The cross-reactions with the other steroids were <0·1%.

The accuracy of the assays was determined after adding varying concentrations of the steroid hormones to plasma. The linear regression analysis of the data gave $y = ax + b$ (y = pg/ml found, x = pg/ml added) and the correlation coefficient, r. For the individual assays the values for a, b and r were 0·98, 72 and 0·99 for androstenedione; 0·99, 98 and 0·99 for epitestosterone; 0·98, 295 and 0·98 for testosterone; 0·99, 0·33 and 0·99 for oestrogens. The intra- and interassay coefficients of variation were 6 and 12%, 15 and 16%, 6 and 14% and 7 and 11% respectively. The sensitivity of the assays was 16 pg/ml.

RESULTS AND DISCUSSION

The results of the steroid determinations are shown in Fig. 1. Plasma concentrations of total unconjugated oestrogens increased from 2·00 ± 0·20 to 3·87 ± 0·50 ng/ml during the last week of pregnancy, declined slightly 1 day before parturition, and then fell precipitously, reaching levels below the limit of detection on day 4 *post partum*. These observations are in agreement with those of Stellflug *et al.* (1978) who suggested that the decrease of oestrogens in peripheral blood 1 day before parturition results from a reduced blood-flow through fetal and maternal parts of the placentome. Data obtained by Hoffman *et al.* (1979) indicated that it is the placenta itself and not the fetus which is contributing to the increased oestrogen concentrations before parturition. Assuming active aromatases to be present in the placenta, it would be expected that the immediate precursors of these increased oestrogens are circulating androgens. A markedly increased androgen concentration in the maternal peripheral circulation has been found in the sheep (Steele, Flint & Turnbull, 1976) but not in the goat (Flint & Burrow, 1979).

In our study, however, the peripheral plasma concentrations of androstenedione, epitestosterone and testosterone remained approximately constant during the last week of

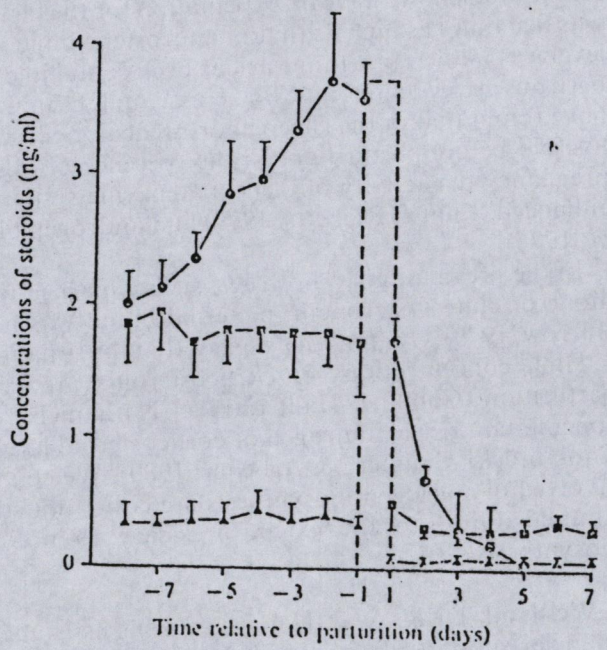

Fig. 1. Concentration of oestrogens (○), androstenedione plus epitestosterone (□) and testosterone (▲) in the peripheral plasma of cows during the week before and the week after parturition, shown by dotted lines (means ± S.E.M.; n = 8).

pregnancy, although great variations existed between individual animals. A rapid decline in the levels of all three androgens occurred, similar to that in the levels of oestrogen, to yield very low post-partum values from the day before to the day after parturition (see Fig. 1).

There are few other reports concerning androgen levels in peripheral blood of cows during the late stages of pregnancy. Hoffman *et al.* (1976) reported a concentration of 0·33 ng testosterone/ml in the blood of cows at about day 270 of pregnancy. Mongkonpunya, Lin, Noden, Oxender & Hafs (1975) found, in serum from the jugular vein of heifers on day 260 of pregnancy, concentrations of testosterone between $0·36 \pm 0·19$ ng/ml for those with female and $0·46 \pm 0·12$ ng/ml for those with male fetuses. In our study, the testosterone concentration in peripheral blood of cows before parturition was $0·40 \pm 0·20$ ng/ml (the sex of the fetus was not considered) and after parturition, only low levels of about $0·07 \pm 0·01$ ng/ml were present. The androstenedione concentration in jugular vein serum was reported by Mongkonpunya *et al.* (1975) to be $0·73 \pm 0·11$ ng/ml for heifers with a female and $1·02 \pm 0·13$ ng/ml for those with a male fetus. We found androstenedione concentrations of $0·90 \pm 0·18$ ng/ml in the blood of cows during the last week of pregnancy and levels around the detection limit of our assay (50 pg/ml) after parturition.

However, a rapid conversion of androstenedione to other metabolites, particularly epitestosterone, can take place in bovine blood after its collection (Bamberg *et al.* 1978; Möstl, Choi & Bamberg, 1980) and therefore the accurate estimation of plasma levels of androstenedione presents difficulties. For example, we have found the half-life of androstenedione *in vitro* to be about 6 min in blood samples taken from cows at various times during the oestrous cycle and during pregnancy (Bamberg *et al.* 1978; Möstl, E., 1978; Möstl, K., 1978). Although we inhibited the in-vitro conversion by the addition of methanol to the blood samples (see Methods), we measured both androstenedione and epitestosterone simultaneously, to provide a more realistic picture of androstenedione levels *in vivo*.

It was found that, similarly to androstenedione, the epitestosterone levels remained approximately constant at $0·92 \pm 0·08$ ng/ml during the last week of pregnancy and declined sharply after parturition to $0·32 \pm 0·03$ ng/ml. It is noteworthy that the levels of this major metabolite of androstenedione were of the same order as the levels of androstenedione itself. At the moment we have no explanation for the fact that the concentration of epitestosterone was five times as high as that of androstenedione *post partum*. However the epitestosterone level was in the same range as has been found in cows during the pro-oestrous phase of the oestrous cycle (Bamberg, Choi, Hassaan, Kläring, Möstl & Stöckl, 1980). Steele *et al.* (1976) have found that in the sheep androstenedione levels in the utero-ovarian vein rise three-to fivefold before parturition. In the presence of high levels of corticosteroids at the end of pregnancy the activity of placental enzyme systems involved in androgen biosynthesis are enhanced (Flint & Ricketts, 1979) and androgen biosynthesis is increased thereby (Steele *et al.* 1976).

In the pregnant goat, however, such a marked increase in concentrations of androstenedione or epitestosterone in maternal blood was not observed before parturition (Flint & Burrow, 1979). Our findings suggest that the situation in the cow is similar to that in the goat.

High concentrations of corticosteroids are present in the bovine fetus just before parturition (Comline, Hall, Lavelle, Nathanielsz & Silver, 1974), but we did not find a concomitant rise in androgen or corticosteroid levels in the maternal blood (Möstl, Smolle, Choi, Stöckl, Bamberg & Arbeiter, 1980). We conclude that the placental enzyme systems involved in androgen biosynthesis are either not stimulated by fetal corticosteroids or are stimulated just to the extent necessary to provide enough precursors for oestrogen biosynthesis.

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