Estrone Concentrations in the Peripheral Blood of Pregnant Cows

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Abstract

Blood estrone concentrations of 65 pregnant dairy cows were measured by an Ittrich-Kober fluorometric procedure. Average estrone increased slowly from 1.2 ng/ml at 16 to 14 weeks prepartum to 2.5 ng/ml at 8 to 6 weeks prepartum, and then more rapidly to 4.8 ng/ml at 4 to 2 weeks before parturition. The highest average, 8.3 ng/ml, was 5 days prepartum; no samples were taken closer to parturition. The lowest average (0.7 ng/ml) was 5 days postpartum.

Introduction

Estrogenic activity in bovine urine (5-11) and faecal material (5) increases throughout pregnancy. Erb et al. (6) have shown that the major urinary steroid, estradiol-17a, increased tenfold near term. Concurrent levels of estrone and estradiol-17β increased only fourfold and showed no change.

Reports on estrogenic activity in bovine blood as determined by biological assays have been highly variable. Bitman et al. (1) reported 0.7 μg estradiol-17β equivalents per liter peripheral blood at 6 to 8 months of pregnancy. In contrast, Szego and Roberts (16) reported 3.7 μg estradiol-17β equivalents per liter blood at 6 months of pregnancy. Pope et al. (12) indicated that most of the estrogenic activity of the phenolic fraction of bovine peripheral blood was due to estrone. Their mouse uterine weight bioassay detected 7 μg equivalents of estrone per liter at 7 to 9 months, 1 μg at 5 to 6 months and none at 2 to 3 months of pregnancy.

Increasing estrogenic activity in the blood of the cow near term supports the theory that parturition may be initiated by decreasing progesterone and increasing estrogens. Caspo (4) has demonstrated that progesterone “blocks” uterine contraction. Short (15) reported that progesterone of bovine blood remained constant from 32 to 256 days of pregnancy and then decreased rapidly just before calving. Erb et al. (6) have questioned whether blood progesterone always declines before parturition in the cow. Failure of progesterone to decrease may be overcome by increasing estrogen in the blood. On the assumption that uterine contractility reflects estrogen levels, Schofield (14) reported that blood estrogen in the rabbit is low during the first half of pregnancy, begins to increase at mid-term and is high at parturition.

The purpose of our study was to determine chemically the steroid estrogen concentrations in the blood of cows during pregnancy. Our data relate to estrone concentrations in the peripheral blood from about 4 months before until 5 days after parturition.

Experimental Procedure

The blood samples were from 49 Holstein cows (500 to 655 kg) and 16 Ayrshire cows (382 to 545 kg), at from 4 months before until 5 days after parturition. All the cows had calved previously. The samples were drawn by syringe from the external jugular vein. Each sample (20 ml) was immediately dispersed in 440 ml 1.5 M HCl to prevent enzymatic changes and to provide a dispersion ready for hydrolysis. This dispersion was divided into 2 equal portions for hydrolysis to obtain duplicate analytical results.

Each subsample was then processed by Brown's (2) procedure but with these modifications: a) the reflux time was shortened from 60 to 45 minutes; b) stable emulsions forming during the first extraction with diethyl ether were broken by centrifuging at 1300 × g for 5 minutes; c) the dark mass between the aqueous and organic layers was washed with diethyl ether and then discarded; the remaining liquid was returned to the separatory funnel and the extraction continued; and d) the pooled ether extracts were washed once with 8% NaHCO₃ (100 ml) and once with water (25 ml), and extraction with water for removal of estriol was omitted.

The ether was removed under reduced pressure in a rotatory evaporator. The residue was

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dissolved in 1.0 ml absolute ethanol and transferred to a separatory funnel (125 ml). Twenty-five milliliters of 1.6% NaOH was added and then 25 ml of benzene, followed by 25 ml of light petroleum, the evaporation flask being rinsed out successively with benzene and petroleum before they were added to the separatory funnel. After vigorous shaking, the separatory funnel was centrifuged at 1200 × g to break the emulsion. The alkaline phase was drawn into the methylation flask (125 ml ground stoppered Erlenmeyer). The organic phase was re-extracted further with 25 ml 1.6% NaOH, and the separatory funnel shaken and centrifuged as before. The alkaline phase was drawn into the methylation flask and the organic phase was discarded.

To the methylation flask were added 0.9 g boric acid and 1.0 ml dimethyl sulfate and the flask was shaken in a water bath at 37°C until the dimethyl sulfate had dissolved (about 10 minutes). Two milliliters of 20% NaOH and 1.0 ml dimethyl sulfate were added, and the flask was shaken as before and remained at 37°C for 1 hour. The flask was removed from the bath and 10 ml of 20% NaOH was added to destroy remaining dimethyl sulfate, then 2.5 ml of 30% H₂O₂ was added and the solution transferred to a separatory funnel. The methylation flask was rinsed with 25 ml light petroleum added to the separatory funnel. After shaking, the aqueous alkaline was discarded. The organic phase was washed 3 times with 5 ml water each time, care being taken to drain the water thoroughly. The organic phase was decanted from the separatory funnel into a flask (100 ml) and then applied to a column of alumina (Woelm) prepared and standardized as described by Brown (2). Elution of the fractions was as described by Brown (2).

After chromatography the fraction containing estrone was evaporated under a stream of nitrogen and stored at −10°C pending measurement of the estrone by the Ittrich-Kober procedure described by Brown et al. (3). This procedure was followed in detail except that, first, the reaction was performed in one tube and, second, after removal of the aqueous phase by aspiration, the tetrachloroethane phase was transferred to a separate tube for fluorometry. Fluorometry was in a Turner photofluorometer, Model 110, fitted with a high sensitivity conversion unit no. 110-386. This unit was equipped with a microcuvette adapter no. 110-386 so that measurements could be made with as little as a 0.5 ml sample. The primary filter was no. 110-829 (535 μm). The secondary filter was no. 110-819 (560 μm). Fluorescence was read as soon as practicable because it faded with time. Standards and blanks were run with each set of fluorimetric determinations to correct for fluorescence from the reagents in the Ittrich-Kober procedure. Analyses on bull's blood and blood from cows at 3 months of pregnancy revealed no estrone, thus showing that the solvents used before the Ittrich-Kober procedure did not introduce interfering fluorescence. The standards contained 0.05 μg and 0.10 μg estrone-3-methyl ether and were prepared anew every 2 to 3 weeks and stored at −10°C.

To test the accuracy of the analytical procedure, 13 blood samples were obtained from both pregnant and nonpregnant cows. The dispersion of each sample was divided into 2 portions as before. After hydrolysis, 0.4 μg estrone was added to one portion while the corresponding portion served as a blank. The analyses were then completed. Recovery of the added estrone was 71.7% (SE = ±2.54 for 12 df). This is slightly higher than Roy's (13) recovery of 61 ± 5% for estrone added after hydrolysis. Since Brown (2) and Roy (13) have demonstrated a loss of approximately 15% of the estrone originally present during acid hydrolysis, the overall recovery becomes 0.85 × 0.71 × 100 = 61%. Accordingly, our analyses have been corrected for 61% recovery during the analytical procedure.

Precision of the method was estimated by picking 20 duplicate analyses at random from all available data for pregnant cows. These values were from 0.52 to 14.3 ng/ml. The standard deviation (calculated from SD = \sqrt{\sum d^2/N}) was ±0.045 ng/ml for N = 20. The corresponding confidence limits (P = 0.01) were ±0.42 ng/ml.

The chromatographic fractions correspond—

![Fig. 1. Individual blood estrone concentrations of cows before and after parturition.](image-url)
ing to estradiol-17β were also analyzed for a number of samples. The values were either zero or very low. We concluded that the procedure was inadequate for quantitation of the estradiol-17β in the peripheral blood of pregnant cows.

Results and Discussion

The analyses, corrected for methodological losses, are presented in Fig. 1 and summarized in Table 1. Uniformity of variance could not be assumed for the data as a whole. Accordingly the significance of the differences between pairs of means was examined by a modified Student's "t" test which allows for nonuniformity of variance (17).

Blood estrone increased slowly to an average of 2.5 ng/ml at 8 to 6 weeks prepartum and then more steeply to 4.8 ng/ml at 4 to 2 weeks prepartum. The average at 5 days prepartum was higher (P < 0.05) than that for any other group. The highest individual value of 14.3 ng/ml was in this group.

Hunter et al. (7) reported recently that the excretion of total estrogens and estradiol-17α increase from Day 34 prepartum to parturition. At parturition cows having gestations of 280 to 286 days excreted more total estrogens principally as estradiol-17α than cows having shorter or longer gestations. The major increase in urinary estrone occurred before 2 weeks prepartum. After 2 weeks prepartum the average urinary estrone level did not increase and was not influenced by the length of gestation. Differences between our results and those reported by Hunter et al. (7) indicate that the amount of estrone excreted in the urine does not necessarily reflect the amount in blood. However total urinary estrogen may parallel blood estrogen concentrations.

We concluded that estrone in blood of pregnant cows increases rapidly during the last month of pregnancy. In our work the highest average was 5 days before parturition, but from the observed averages (Fig. 1), it seems probable that peak blood estrone in a given cow may occur sometime between 5 days prepartum and parturition itself. The data are not in conflict with the theory that parturition may be initiated by increased estrogens in blood and decreases in progesterone.

It would be of interest to determine the concentrations of estrone, estradiol-17β, and estradiol-17α in the blood of cows more closely over the week before and after parturition, especially as Mellin, Erb and Estergreen (10) have noted a significant increase of urinary estrogen excretion during 40 hours before parturition.

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