Urinary Estrogen Excretion Rates During Pregnancy in the Bovine

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Abstract

Urine was collected from 48 cows from 101 to 285 days of pregnancy, to define urinary excretion rates of estrone, 17β-estradiol, 17a-estradiol, and an incompletely identified triol, hereafter referred to as estriol. Enzyme hydrolysis was used as the first extraction step to minimize destruction of 17α-estradiol. The estrogens were separated and purified by paper partition chromatography and measured fluorimetrically. Breed, age, and live weight of the cow were not significantly related to excretion rate when the latter was adjusted to a 500-kg basis (μg/hr × live weight × 500 kg). Average total estrogen excretion ± one standard error per hour per 500 kg during pregnancy was as follows: 101 to 123 days—300 ± 96 μg; 165 to 175 days—581 ± 154 μg; 226 to 237 days—691 ± 104 μg; 250 to 254 days—1,809 ± 580 μg; and 271 to 285 days—3,402 ± 412 μg. The latter two means were significantly higher than means of each of the other periods. Estriol excretion was highest during 250 to 254 days (P < 0.01, except for 226 to 237 days). Changes in 17β-estradiol were not significant, but estrone increased significantly (P < 0.01) by 271 to 285 days and 17α-estradiol increased significantly (P < 0.01 by 250 to 254 days. Excretion rate of estrone tended to be higher or equal to 17α-estradiol through 237 days, but 17α-estradiol increased tenfold thereafter, compared with a fourfold increase for estrone.

As recently reviewed, relatively large quantities of estrone and 17α-estradiol are present in bovine urine during pregnancy (12). Also, generally minor amounts of 17β-estradiol and a fourth metabolite tentatively identified as 1,3,5(10)-estraatriene-3,16β,17α-triol are present following intravenous administration of estrone-16α-C (9). This latter compound will be subsequently referred to as estriol, since it appears rather definitely a member of the triol group of endogenous estrogen compounds (9, 14, 16).

Variations in methods used, especially those aspect involving hydrolysis purification, and specificity of the quantitative step, have resulted in large differences between laboratories relative to the reported amount of estrogens excreted in bovine urine during pregnancy (12). Formerly, hot acid was used to hydrolyze bovine urine for variable periods of time prior to extraction of the estrogens. It was subsequently shown that 17α-estradiol was especially acid-labile and that yields of this metabolite were increased if an enzyme hydrolytic procedure was used prior to initial extraction with ether (7, 9, 14, 18). It seems clear that urinary estrogen excretion rate increases rapidly during the last one-half of bovine pregnancy (12) and that a further significant increase occurs during the 40-hour period preceding parturition (15). The magnitude of the changes during pregnancy is not clear, because of the different methods employed (12, 15). It was our purpose to measure urinary estrogen after 100 days of pregnancy, to study changes in excretion rate of the metabolites using a method that minimized destruction of 17α-estradiol. A brief report has been made using the data unadjusted for body weight (5).

Experimental Procedure

Urine was collected during 1961-65 at 8-hour intervals for 24-hour periods from 12 Guernsey, 28 Holstein, and 8 Jersey cows from 101 to 285 days of pregnancy, using the harness and bag apparatus designed by Gorski et al. (8). Urine was collected from six cows one to two days preceding parturition, four cows prior to sacrifice at 250 to 258 days, and 38 cows preceding ovarietomy at 103 to 251 days. At each 8-hour interval urine volume was re-
corded and a constant fraction or aliquot was filtered through spun glass-wool. The aliquot was boiled for ten minutes and held at 5 C until the end of the collection period for each cow. Then a 24-hour aliquot was prepared, frozen, and stored at -10 C until assayed. In some cases, the samples were stored as 8-hour aliquots until assayed. Initially, assays were done at Washington State University and then transferred to Purdue University in 1962. Urine samples requiring shipment were transported by air express in a frozen state in the presence of solid CO₂.

The urine was assayed for estrone, 17 β-estradiol, 17 a-estradiol, and an estriol, using the method of Mellin et al. (14). Proof of identification of the compounds and precision of the method have been reported in detail (7, 13, 14). In brief, before hydrolysis trace amounts of estrone-16.14C and 17 β-estradiol-14C were added to the urine for the purpose of estimating method losses. Due to chemical similarity, recovery of the latter compound was used to estimate method losses for 17 a-estradiol. No attempt was made to estimate the losses of estriol due to method, because the exact chemical configuration was not definitely known (9, 14, 16) and radioactive triols were not commercially available. Enzyme (β-glucuronidase) was used for initial hydrolysis, followed by acid hydrolysis, to minimize loss of 17 a-estradiol and to maximize total yield of estrogen extracted (7, 9, 14, 18). Following partial purification, the estrogens were isolated, using paper partition chromatography, measured fluorimetrically, and corrected where appropriate for method losses and quantity of added radioactive standards. The corrected concentration of each estrogen was multiplied by the volume of urine voided during the time period represented by the aliquot assayed, and divided by the number of hours involved to establish the average excretion rate per hour.

Statistical techniques included step-wise regression using both the build-up and tear-down models (1, 17), analysis of variance (17), and a multiple range test for determining differences between means with unequal numbers of replications (10).

Results and Discussion

Variables affecting statistical interpretations. The estrogen measurements were initially analyzed to test for linear, quadratic, and cubic relationships during pregnancy, independent of breed, age, and live weight of the cow, using a tear-down, step-wise regression computer program. This approach was unsuccessful, largely because excretion rate increased gradually from four to eight months and then accelerated (Fig. 1). Attempts to describe this change by various transformations were unsuccessful.

Since averages for periods between 101 and 237 days and between 250 to 285 days appeared to be essentially linear with days of pregnancy, the two groups were analyzed separately. A build-up, step-wise regression program (1) was used, with the estrogen data both unadjusted and adjusted for live weight of the cow (hourly excretion rate ÷ live weight in kg × 500 kg). The latter procedure was the most satisfactory for the purposes of this analysis, since the partial regressions between urinary estrogen excretion rate per hour per 500 kg, and breed, age, and weight of the cow did not approach significance (P < 0.10). In the data unadjusted to a 500-kg live weight basis, age of the cow was not a significant factor, but breed, stage of pregnancy, and live weight were confounded. Weight and stage of pregnancy were the two most important variables related to estrogen excretion rate in the data unadjusted to a 500-kg basis.

Excretion rate (µg/hr/500 kg). The averages and standard errors for excretion rates for the four estrogens and their total for six periods of pregnancy are shown in Figure 1. The two minor metabolites at each stage of pregnancy shown in Figure 1 were 17 β-estradiol and estriol. The former did not vary significantly (P < 0.10), but the period averages for estriol were significantly different (P < 0.01). Estriol excretion rate, 202 µg/hr/500 kg at 251 days, was significantly (P < 0.01) higher than during the other periods, except 230 days (Fig. 1). Excretion of estrone was variable from one period to the next and did not show a significant (P < 0.01) rise until the 281-day period of pregnancy (Fig. 1). Though estrone excretion increased from 128 ± 15 µg/hr at the 106-day period to 510 ± 143 µg/hr at the 251-day period of pregnancy, one cannot be certain of the significance, because of the variation between cows.

Excretion of 17 a-estradiol was relatively low through the 230-day period, but increased approximately fourfold by 251 days, as compared to the mean of the previous three periods and more than doubled again near the end of pregnancy (P < 0.01). Likewise, total estrogen excretion, though increasing from 106 to 230 days, was significantly (P < 0.01) higher during the 281-day period than during any of the preceding periods. At 251 days, total estrogen
excretion was significantly higher as compared to 106- (P < 0.01) and 170-, 205-, and 230- (P < 0.05) day periods of pregnancy.

**General discussion.** The most striking feature of these data is the sharp increase in excretion of estrone and 17α-estradiol, especially the latter, during the last one month of pregnancy. Moreover, Mellin et al. (15) observed an additional 29% increase during the 40-hour period preceding parturition. The excretion rate during the 8-hour interval including parturition was 4,280 ± 695 µg/hr converted to a 500-kg live weight basis. This compares to 3,402 ± 412 µg/hr at 281 days and 1,809 ± 580 µg/hr at 251 days of pregnancy. Excretion of total estrogen during the 106-day period was approximately three times greater than a 90 µg/hr rate reported for one 442-kg Holstein heifer one day before estrus (13). The failure to observe a greater change in excretion rate from the 170-day period through the 230-day period is not understood. However, Veenhuizen et al. (19) reported that concentration of total estrogen in fetal cotyledons during pregnancy increased more between the periods 230 to 237 days (1.59 µg/100 g) and 256 to 275 days than between the periods 102 to 158 days (1.03 µg/100 g) and 230 to 237 days.
Furthermore, a threefold increase was observed for fetal cotyledons sampled after parturition (19). The total estrogen present in the fetal cotyledons increased tenfold from 102 to 275 days of pregnancy, due primarily to increased weight of the fetal cotyledons (19). Therefore, increases in urinary estrogen during pregnancy appear to coincide with increases in concentration of estrogens in the fetal cotyledons.

The relatively low urinary excretion rate from 200 to 237 days may partially explain why cows do not ordinarily abort soon after ovariectomy during this period (6). Since there is evidence that extraovarian sources of progesterone are near a maximum during the 200- to 237-day period (3), the amount available may be sufficient to prevent sudden dominance from estrogen, thereby avoiding abortion. This aspect is covered in more detail in a companion paper (4). These interpretations of urinary estrogen excretion rate assume near-constant ratios between feces and urine. It has been shown that more than one-half of the estrogen is excreted in the feces in the nonpregnant (9, 12) and pregnant (2, 11, 12) cow. The ratio of \( ^{14} \text{C} \) in feces and urine was nearly the same after administration of two dose levels of estrone-16-\( ^{14} \text{C} \) during the luteal phase of the nonpregnant heifer (9), and levels increase in both feces and urine during pregnancy (2).

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References


