URINARY OESTROGEN EXCRETION IN THE PIG DURING PREGNANCY AND PARTURITION

J. I. RAESIDE

Department of Physiological Sciences, Ontario Veterinary College, Guelph, Canada

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Summary. The daily urinary excretion of oestrogens was measured by a chemical method, at weekly intervals through the greater part of gestation in two sows and during the last 6 weeks in four other sows. Oestrone appeared to be the principal component of the oestrogens in pregnant sows' urine. After an early peak in the 4th week from conception, the level of oestrone remained low until about the 10th week and then rose steadily to high levels at term.

Complete and continuous collection of urine, uncontaminated with faeces, was made from two sows for several days before parturition and from a total of five sows during, and for a few days after, parturition. A rapid decrease in urinary oestrogen excretion was seen after the sows had farrowed.

INTRODUCTION

Oestrogens were found at two stages of gestation in sows when earlier attempts were made to use biological and chemical tests for the detection of pregnancy (Cowie, 1948). This peculiar pattern of urinary oestrogen excretion in the pregnant sow was confirmed later by Bredeck & Mayer (1958), who described the chemical determination of oestrone and oestriol. Velle (1958, 1959), using the chemical method of Brown (1955), was unable to find any appreciable amounts of oestriol or oestradiol-17 β ,* even in late pregnancy. He concluded that oestrone is the only oestrogenic hormone excreted in significant quantities during pregnancy in this species. A similar conclusion was reached recently by Rombauts (1962), who also used Brown's method, with enzymic hydrolysis. The results reported in the present paper support the view of Velle, as to the qualitative nature of the urinary oestrogens, but provide evidence of much greater amounts of oestrone in the urine of the sow in late pregnancy.

A method for the chemical estimation of oestrogens in pigs' urine was used (Raeside, 1963a), with some simplification for greater amounts of oestrogen. Complete collections of urine, free from contamination with faeces, allowed expression of the results as daily excretion of urinary oestrogen during and after parturition, as well as in pregnancy.

* Referred to subsequently as oestradiol.

MATERIALS AND METHODS

Most of the animals used were mature sows of the Large White breed. One Landrace gilt (Sow 1) and a mature, Landrace $\times$ Large White sow (Sow 10) were included.

Urine was collected by means of an indwelling catheter in the bladder (Raeside, 1963b). For the greater part of gestation in two sows (Sows 1 and 2), 24-hr collections were made at approximately weekly intervals. Four sows (Sows 3, 4, 5 and 6) contributed similarly, in late pregnancy only. Continuous collection of urine was carried out with two sows (Sows 8 and 10) for 2 to 3 weeks before farrowing. A total of five sows (Sows 5, 7, 8, 9 and 10) farrowed with the catheter retained in the bladder, and so provided material for estimations of urinary oestrone excretion during parturition. The litters were removed at birth from these same five sows and urine collection continued for several days. No adverse effects were noted in any of the animals. The sow catheterized for the longest period (Sow 8) delivered fourteen live piglets in a normal parturition. Litter sizes ranged from nine to eleven for the other four sows farrowing with the catheter in place.

The quantitative estimation of urinary oestrogens was done chemically by a much simplified version of a procedure described recently in detail (Raeside, 1963a). Aliquots of 10 ml were taken, in duplicate, from daily urine samples and diluted to 100 ml with water before acid hydrolysis. The ether extract was purified by simple solvent partition and a convenient amount taken for a Kober reaction (Bauld, 1956). Kober chromogens were extracted with tetrachloroethane (Salokangas & Bulbrook, 1961), containing *p*-nitrophenol, and optical density readings made with a Unicam SP 600 spectrophotometer. Recoveries were about 90 % with 5 μ g of oestrone added, before hydrolysis, to urine from ovariectomized sows. The results presented below were not corrected for losses.

For samples from sows in early pregnancy, aliquots of 25 ml of urine were taken and diluted to 200 ml with water. An additional saponification step (Bauld, 1956) was included for further purification. Estimations on samples found to contain less than 100 μ g per litre were repeated using the full procedure for non-pregnant sows' urine (Raeside, 1963a).

RESULTS

Preliminary studies had involved a separation of the oestrone and oestradiol fractions by partition chromatography on a Celite column. The amounts of oestradiol found were very small, even in late pregnancy. Oestriol was not detected in larger aliquots of urine from several samples taken at different stages of pregnancy. With enzymic hydrolysis, other Kober positive materials were detected in amounts less than 10 % of the total urinary oestrogens (J. I. Raeside, unpublished data). Since their contribution to the readings from an acid hydrolysis would be even less, the estimations for urinary oestrogens were considered to be for oestrone.

Results were obtained throughout most of pregnancy for Sows 1 and 2, as shown on a logarithmic scale in Text-fig. 1. In both sows, a characteristic pattern

of urinary oestrogen excretion was seen in which an early peak was followed by low levels and, then, a rapid increase towards term. Data from four additional sows in late pregnancy are included also. The results for Sow 5 are shown for a few days after farrowing, as well. Urinary oestrogen levels in late pregnancy were obtained in more detail for Sows 8 and 10 (Text-fig. 2).

Levels during, and after, parturition were determined for a total of five sows, as shown on a logarithmic scale in Text-fig. 2. Data from Sows 7 and 9 are

TEXT-FIG. 1. Daily urinary oestrogen excretion by pregnant sows. ● Sow 1; ■ Sow 2; ○ Sow 3; □ Sow 4; ▲ Sow 5; △ Sow 6.

of particular interest. Some piglets were delivered in one urine-collection period while the remaining litter-mates arrived early in the next period. For Sow 7, an opportunity was presented to estimate oestrone for a 12-hr collection of urine on either side of parturition. Urinary oestrone levels continued to rise until farrowing. The values given for Sow 10 on the 2 days immediately before parturition had to be based on a 48-hr urine collection.

After delivery the urinary oestrogen levels fell rapidly, and amounts normal for the non-pregnant animal were reached within 5 days.

DISCUSSION

From the results obtained in this investigation, it is suggested that oestrone is the only urinary oestrogen excreted in significant quantities by the sow in late pregnancy. The data are in agreement with the previous findings of Velle (1959) and of Rombauts (1962), and in conflict with those of Bredeck & Mayer (1958), who recorded the presence of oestriol in greater amounts than oestrone. It is difficult to find an explanation for this discrepancy in view of the high concentrations of oestrogens involved.

The data for early pregnancy, from Sows 1 and 2, support the more complete investigation of urinary oestrogen levels made by Lunaas (1962), with three sows. Rombauts (1962) also reported similar findings for three sows in early pregnancy. In one animal he was able to show that the levels remained low,

TEXT-FIG. 2. Daily urinary oestrogen excretion in late pregnancy, during parturition and early *post partum*. The arrows show the time of farrowing for each sow.

after the early peak, until about the 80th day of gestation. Thereafter, the amounts of urinary oestrone increased rapidly to reach a plateau which was maintained until the last days of gestation when a second clear peak was found. Data from all ten sows in late pregnancy in the present study show a more regular, yet steep, rise which continued up to the time of farrowing. The absence of a plateau is best illustrated by the pattern from the continuous collection of urine from one sow for 3 weeks before farrowing. Since it is most likely that the values given by Rombauts (1962) and those obtained in the present work were for oestrone alone, it is difficult to suggest an explanation for the slight deviation in the pattern of urinary oestrogen excretion exhibited by his sow. It may be noted here that the peak value from his animal at parturition was at the lower end of the range recorded for our sows at farrowing.

The source of the large quantities of oestrone in the urine of the sow during the last 2 weeks of pregnancy is not known, but the rapid decrease following delivery would seem to implicate the placentae as major sites of oestrogen production. Unfortunately, the placental weights were not taken. There was no apparent relation between oestrogen excretion and the number of piglets delivered at term.

The complete collection of urine during farrowing in five animals provided a unique opportunity to estimate urinary oestrogen levels in relation to parturition in the sow. There was no evidence of a fall in the output of oestrogen until the day of farrowing. Even then, the amounts of oestrogen differed little in two 12-hr collections of urine from one sow at parturition. The extent to which these urinary oestrogen levels reflect oestrogen concentrations in blood remains to be determined.

After farrowing, the amounts of oestrogen in the urine decreased rapidly, as was noted by Rombauts (1962). The rapid elimination of oestrogens by the sow after parturition resembles more closely the pattern of urinary oestrone, rather than of oestriol, for women after delivery (Brown, 1956).

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